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November 24, 1999

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Assistant Commissioner for Patents
Washington, D.C. 20231

Box Patent Application

Re: U.S. Non-Provisional Utility Patent Application under 37 C.F.R. § 1.53(b)
Appl. No. To Be Assigned; Filed: Herewith
For: **Death Domain Containing Receptor-4 Antibodies (As Amended)**
Inventors: NI *et al.*
Our Ref: 1488.1300004/EKS/EJH

Sir:

The following documents are forwarded herewith for appropriate action by the U.S. Patent and Trademark Office:

1. PTO Utility Patent Application Transmittal Form (PTO/SB/05);

2. U.S. Utility Patent Application entitled:

Death Domain Containing Receptor-4 Antibodies (As Amended)

and naming as inventors:
NI, Jian
ROSEN, Craig A.
PAN, James G.
GENTZ, Reiner L.
DIXIT, Vishva M.

the application consisting of:

a. A specification containing:

   (i) 63 pages of description prior to the claims, including a sequence listing on pages 47 to 63;

   (ii) 8 pages of claims (21 claims);

   (iii) a one (1) page abstract;

b. 8 sheets of drawings: (Figures 1A, 1B, 2A, 2B, 3, 4A, 4B, 5A, 5B, 5C, 6A & 6B);

c. Substitute Sequence Listing (pages 1-16);

d. A substitute computer readable disk copy of the Substitute Sequence Listing;

e. A copy of the executed Declaration, as filed in U.S. Appl. No. 09/013,895;

f. Preliminary Amendment with attachments:
   - Exhibit A - *Gibco BRL Products and Reference Guide, 2000-2001*;
   - Exhibit B - Ausubel *et al.*, *Current Protocols in Molecular Biology*;
   - Exhibit C - Abstract of Chou and Roizman, *J. Virol. 57*:629-637;
   - Exhibit D - Abstract of Mauri *et al.*, *Immunity* 8:21-30;

g. Our check No. 26047 for $1,594.00 to cover:
   $760.00 - filing fee for patent application;
   $834.00 - fee for excess claims;

3. PTO Fee Transmittal Form PTO/SB/17 *(in duplicate)*;

4. Authorization to Treat a Reply As Incorporating An Extension of Time Under 37 C.F.R. § 1.136(a)(3) *(in duplicate)*; and

5. Two (2) return postcards.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the substitute sequence listing and the computer readable copy of the sequence listing submitted herewith are the same.

It is respectfully requested that, of the two attached postcards, one be stamped with the filing date of these documents and returned to our courier, and the other, prepaid postcard, be stamped with the filing date and unofficial application number and returned as soon as possible.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036. A duplicate copy of this letter is enclosed.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Eric K. Steffe
Attorney for Applicants
Registration No. 36,688

EKS/EJH/cmg
Enclosures

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Please type a sign(+) inside this box → ⊞

PTO/SB/05 (2/98)
Approved for use through 09/30/2000 OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

UTILITY PATENT APPLICATION TRANSMITTAL *(Only for new nonprovisional applications under 37 CFR § 1 53(b))*		
	Attorney Docket No.	*1488.1300004/EKS/EJH*
	First Inventor or Application Identifier	*NI et al.*
	Title	*Death Domain Containing Receptor-4 Antibodies (As Amended)*
	Express Mail Label No.	

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

1. ☒ * Fee Transmittal Form (*e.g.*, PTO/SB/17)
(Submit an original, and a duplicate for fee processing)

2. ☒ Specification [Total Pages 72]
(preferred arrangement set forth below)
- Descriptive title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings *(if filed)*
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 8]

4. ☒ Oath or Declaration [Total Pages 4]

a. ☐ Newly executed (original or copy)

b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 17 completed)*
[Note Box 5 below]

i. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR §§ 1 63(d)(2) and 1.33(b).

5. ☒ Incorporation By Reference *(useable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein

6. ☐ Microfiche Computer Program *(Appendix)*

7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*

a. ☒ Computer Readable Copy

b. ☒ Paper Copy (identical to computer copy)

c. ☒ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8 ☐ Assignment Papers (cover sheet & document(s))

9. ☐ 37 CFR 3 73(b) Statement (when there is an assignee) ☐ Power of Attorney

10. ☐ English Translation Document *(if applicable)*

11 ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations

12 ☒ Preliminary Amendment

13. ☒ Return Receipt Postcards (2) (MPEP 503)
(Should be specifically itemized)

14. ☐ *Small Entity Statement(s) *(PTO/SB/09-12)* ☐ Statement filed in prior application, Status still proper and desired

15 ☐ Certified Copy of Priority Document(s)
(if foreign priority is claimed)

16 ☒ Other: 37 C.F.R. § 1.136(a)(3) Authorization

☒ Other Substitute Sequence Listing (pp. 1-16); Substitute Computer Readable Disk,

**NOTE FOR ITEMS 1 & 14 IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R § 1 27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R §1 28).*

17. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in a preliminary amendment*
☐ Continuation ☒ Divisional ☐ Continuation-in-Part (CIP) of prior application No: 09/013,895
Prior application information: Examiner Kaufman, C. Group/Art Unit: 1646

18. CORRESPONDENCE ADDRESS

☐ ***Customer Number or Bar Code Label*** ***(Insert Customer No. or Attach bar code label here)*** ***or*** ☒ ***Correspondence address below***

NAME	STERNE, KESSLER, GOLDSTEIN & FOX P L L C				
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NAME (Print/Type)	Eric K. Steffe	*Registration No. (Attorney/Agent)*	36,688
SIGNATURE	Eric K. Steffe	*Date*	November 24, 1999

Burden Hour Statement this form is estimated to take 0 2 hours to complete Time will vary depending upon the needs of the individual case Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231 DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO. Assistant Commissioner for Patents, Washington, DC 20231.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

NI *et al.*

Appl. No.: To Be Assigned

Filed: Herewith

For: **Death Domain Containing Receptor-4 Antibodies (As Amended)**

Art Unit: To Be Assigned

Examiner: To Be Assigned

Atty. Docket: 1488.1300004/EKS/EJH

Preliminary Amendment

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the application as follows. In support of the amendments, attached hereto are (a) a copy of page 22-24 of the *Gibco BRL Products and Reference Guide, 2000-2001* (Exhibit A); (b) a copy of page 2.10.7 of Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., (1997) (Exhibit B); (c) a copy of the abstract of Chou and Roizman, *J. Virol. 57*:629-637 (1986) (Exhibit C); and (d) a copy of the abstract of Mauri, *et al.*, *Immunity* 8:21-30 (1998) (Exhibit D). Also attached are substitute sheets to amend the paper copy of the Sequence Listing as well as a substitute computer readable copy of the Sequence Listing.

In the Title:

After "Receptor-4" please insert --Antibodies--.

In the Specification:

On page 1, please insert just above the heading "*Field of the Invention*" the following paragraph:

--This application is a divisional application of U.S. Patent Application Serial No. 09/013,985, filed January 27, 1998, which claims benefit of 35 U.S.C. Section 119(e) based on U.S. Provisional Application Serial Nos. 60/035,722, filed January 28, 1997 and 60/037,829, filed February 5, 1997. Each of these disclosures is fully incorporated herein by reference.--

On page 1, lines 10-13, please delete the entire paragraph.

On page 5, line 23, please delete "Figures" and insert therefor --Drawings--.

On page 5, line 24, please delete "FIG. 1 shows the nucleotide and deduced amino acid sequence of DR4" and insert therefor --Figures 1A and 1B show the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of DR4 --.

On page 5, line 29, please delete "FIG. 2 shows" and insert therefor --Figures 2A and 2B show--.

On page 5, line 30, after "DR4" please insert --(SEQ ID NO:2)--.

On page 6 lines 18 and 19, please delete "12301 Park Lawn Drive, Rockville, Maryland 20852" and replace therefor: --10801 University Boulevard, Manassas, Virginia 20110-2209--.

On page 7, line 11, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 7, line 20, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 8, line 27, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 9, line 12, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 9, line 15, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 9, line 33, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 9, line 36, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 10, line 7, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 10, line 14, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 10, line 19, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 10, line 21, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 10, line 24, please delete "FIG. 1" and insert therefor --Figure 1A--.

On page 10, line 26, please delete "FIG. 1" and insert therefor --Figure 1A--.

On page 10, line 27, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 10, line 29, please delete "FIG. 1" and insert therefor --Figure 1B--.

On page 11, line 5, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 11, line 6, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 11, line 7, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 11, line 9, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 11, line 10, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 11, line 11, please delete "Figure 1" and insert therefor --Figure 1B--.

On page 11, line 13, please delete "Figure 1" and insert therefor --Figure 1B--.

On page 11, line 18, please delete "and HTXEY80R (SEQ ID NO:7) both shown in Fig. 4" and replace therefor --, as shown in Figure 4A, and HTXEY80R (SEQ ID NO:7), as shown in Figure 4B--.

On page 11, lines 27-28, please delete "150 mM NaCl, 15 mM trisodium citrate" and insert therefor --750 mM NaCl, 75 mM trisodium citrate--.

On page 11, line 29, please delete "20 g/ml" and insert therefor --20 μg/ml--.

On page 11, line 30, after "65" please insert the degree sign: --°--.

On page 12, line 2, please delete "Figure 1 (SEQ ID NO:1) or Figure 2 (SEQ ID NO:3)" and replace therefor --Figures 1A and 1B (SEQ ID NO:1) or Figures 2A and 2B (SEQ ID NO:3)--.

On page 12, line 4, please delete "Figure 1" and insert therefor --Figure 1B--.

On page 12, line 13, please delete "secretary" ain insert therefor --secretory--.

On page 13, line 7, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 13, lines 9 and 10, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 13, line 13, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 14, line 5, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 14, line 20, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 14, line 34, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 15, line 17, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 21, line 24, please delete "FIG.1" and insert therefor --Figures 1A and 1B--.

On page 22, line 3, please delete "109" and insert therefor --132--.

On page 22, line 6, please delete "109 (C-109)" and insert therefor --132 (C-132)--.

On page 22, line 21, please delete "C-109" and insert therefor --C-132--.

On page 22, line 24, please delete "1-109 where C-109" and insert therefor --24-132 where C-132--.

On page 26, line 6, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 26, line 7, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 26, line 8, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 26, line 14, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 26, line 33, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 27, line 6, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 27, line 8, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 27, line 9, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 27, line 12, please delete "FIG. 1" and insert therefor --Figures 1A and 1B--.

On page 28, line 11, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 28, line 12, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 28, line 14, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 28, line 15, please delete "Figure 1" and insert therefor --Figure 1A--.

On page 28, line 16, please delete "Figure 1" and insert therefor --Figures 1A and 1B--.

On page 28, lines 17 and 18, please delete "Figure 1" and insert therefor --Figure 1B--.

On page 28, line 19, please delete "Figure 1" and insert therefor --Figure 1B--.

On page 30, line 4, please delete the extra period.

On page 30, line 37, please delete the comma.

On page 33, line 7, please delete "yl" and insert therefor --ICP--.

On page 34, line 36, please delete "lymphdtoxin" and insert therefor --lymphotoxin--.

On page 37, line 34, please delete "*E. coll*" and insert therefor --*E. coli*--.

On page 38, line 6, please delete "(SEQ ID NO:9)" and insert therefor --(SEQ ID NO:12)--.

On page 39, line 29, please delete "such as" and insert therefor a period --.--.

On page 41, lines 20-21, please delete "(SEQ ID NO:10)" and insert therefor --(SEQ ID NO:9)--.

On page 41, lines 22-23, please delete "(SEQ ID NO:11)" and insert therefor --(SEQ ID NO:10)--.

On page 42, line 20, please delete "(SEQ ID NO:10)" and insert therefor --(SEQ ID NO:9)--.

On page 42, line 28, please delete "(SEQ ID NO:12)" and insert therefor --(SEQ ID NO:11)--.

Please replace the pages of the Sequence Listing with pages 47-62 of the substitute Sequence Listing submitted herewith, and renumber the subsequent pages containing the claims and the abstract accordingly.

In the Claims:

Please cancel, without prejudice to or disclaimer of the subject matter thereof, claims 1-21.

Please add the following claims 22-83:

--22. An isolated antibody which specifically binds the polypeptide of SEQ ID NO:2.

23. The isolated antibody of claim 22, which specifically binds to the polypeptide of amino acids 24 to 468 of SEQ ID NO:2.

24. The isolated antibody of claim 23, which specifically binds to the polypeptide of amino acids 24 to 238 of SEQ ID NO:2.

25. The isolated antibody of claim 24, which specifically binds to the polypeptide of amino acids 132 to 221 of SEQ ID NO:2.

26. The isolated antibody of claim 24, which specifically binds to the polypeptide of amino acids 35 to 92 of SEQ ID NO:2.

27. The isolated antibody of claim 24 which specifically binds to the polypeptide of amino acids 114 to 160 of SEQ ID NO:2.

28. The isolated antibody of claim 23, which specifically binds to the polypeptide of amino acids 169 to 240 of SEQ ID NO:2.

29. The isolated antibody of claim 23, which specifically binds to the polypeptide of amino acids 239 to 264 of SEQ ID NO:2.

30. The isolated antibody of claim 23, which specifically binds to the polypeptide of amino acids 265 to 468 of SEQ ID NO:2.

31. The isolated antibody of claim 30, which specifically binds to the polypeptide of amino acids 267 to 298 of SEQ ID NO:2.

32. The isolated antibody of claim 30, which specifically binds to the polypeptide of amino acids 330 to 364 of SEQ ID NO:2.

33. The isolated antibody of claim 30, which specifically binds to the polypeptide of amino acids 391 to 404 of SEQ ID NO:2.

34. The isolated antibody of claim 30, which specifically binds to the polypeptide of amino acids 418 to 465 of SEQ ID NO:2.

35. The isolated antibody of claim 30, which specifically binds to the polypeptide of amino acids 379 to 422 of SEQ ID NO:2.

36. The isolated antibody of claim 22, wherein said antibody is polyclonal.

37. The isolated antibody of claim 22, wherein said antibody is monoclonal.

38. The isolated antibody of claim 22, where in said antibody is chimeric.

39. The isolated antibody of claim 22, wherein said antibody is an antagonist of the polypeptide of SEQ ID NO:2.

40. The isolated antibody of claim 22, wherein said antibody is an agonist of the polypeptide of SEQ ID NO:2.

41. A composition comprising the isolated antibody of claim 22, and a carrier.

42. A method of producing the isolated antibody of claim 22, comprising:

(a) introducing an immunogen into an animal; and

(b) recovering said antibody.

43. A method of detecting the polypeptide of SEQ ID NO:2 in a biological sample comprising:

(a) contacting a biological sample with the isolated antibody of claim 22; and

(b) determining the presence or absence of said polypeptide in said biological sample.

44. An isolated antibody fragment which specifically binds to the polypeptide of SEQ ID NO:2.

45. The isolated antibody fragment of claim 44, which specifically binds to the polypeptide of amino acids 24 to 468 of SEQ ID NO:2.

46. The isolated antibody fragment of claim 45, which specifically binds to the polypeptide of amino acids 24 to 238 of SEQ ID NO:2.

47. The isolated antibody fragment of claim 46, which specifically binds to the polypeptide of amino acids 132 to 221 of SEQ ID NO:2.

48. The isolated antibody fragment of claim 46, which specifically binds to the polypeptide of amino acids 35 to 92 of SEQ ID NO:2.

49. The isolated antibody fragment of claim 46, isolated antibody fragment specifically binds to the polypeptide of amino acids 114 to 160 of SEQ ID NO:2.

50. The isolated antibody fragment of claim 45, isolated antibody fragment specifically binds to the polypeptide of amino acids 169 to 240 of SEQ ID NO:2.

51. The isolated antibody fragment of claim 45, isolated antibody fragment specifically binds to the polypeptide of amino acids 239 to 264 of SEQ ID NO:2.

52. The isolated antibody fragment of claim 45, which specifically binds to the polypeptide of amino acids 265 to 468 of SEQ ID NO:2.

53. The isolated antibody fragment of claim 52, which specifically binds to the polypeptide of amino acids 267 to 298 of SEQ ID NO:2.

54. The isolated antibody fragment of claim 52, which specifically binds to the polypeptide of amino acids 330 to 364 of SEQ ID NO:2.

55. The isolated antibody fragment of claim 52, which specifically binds to the polypeptide of amino acids 391 to 404 of SEQ ID NO:2.

56. The isolated antibody fragment of claim 52, which specifically binds to the polypeptide of amino acids 418 to 465 of SEQ ID NO:2.

57. The isolated antibody fragment of claim 52, which specifically binds to the polypeptide of amino acids 379 to 422 of SEQ ID NO:2.

58. The isolated antibody fragment of claim 44, wherein said antibody fragment comprises an Fab fragment.

59. The isolated antibody fragment of claim 44, wherein said antibody fragment comprises an $F(ab')_2$ fragment.

60. The isolated antibody fragment of claim 44, where in said antibody fragment is chimeric.

61. The isolated antibody fragment of claim 44, wherein said antibody fragment is an antagonist of the polypeptide of SEQ ID NO:2.

62. The isolated antibody fragment of claim 44, wherein said antibody fragment is an agonist of the polypeptide of SEQ ID NO:2.

63. A composition comprising the isolated antibody fragment of claim 44, and a carrier.

64. A method of producing the isolated antibody fragment of claim 44, comprising:

(a) introducing an immunogen into an animal; and

(b) recovering said antibody fragment.

65. A method of detecting the polypeptide of SEQ ID NO:2 in a biological sample comprising:

(a) contacting a biological sample with the isolated antibody fragment of claim 44; and

(b) determining the presence or absence of said polypeptide in said biological sample.

66. An isolated antibody which specifically binds the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853.

67. The isolated antibody of claim 66, wherein said antibody is polyclonal.

68. The isolated antibody of claim 66, wherein said antibody is monoclonal.

69. The isolated antibody of claim 66, where in said antibody is chimeric.

70. The isolated antibody of claim 66, wherein said antibody is an antagonist of the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853.

71. The isolated antibody of claim 66, wherein said antibody is an agonist of the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853.

72. A composition comprising the isolated antibody of claim 66, and a carrier.

73. A method of producing the antibody of claim 66, comprising:

(a) introducing an immunogen into an animal; and

(b) recovering said antibody fragment.

74. A method of detecting the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853 in a biological sample comprising:

(a) contacting a biological sample with the isolated antibody fragment of claim 66; and

(b) determining the presence or absence of said polypeptide in said biological sample.

75. An isolated antibody fragment which specifically binds to the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853.

76. The isolated antibody fragment of claim 75, wherein said antibody fragment comprises an Fab fragment.

77. The isolated antibody fragment of claim 75, wherein said antibody fragment comprises an $F(ab')_2$ fragment.

78. The isolated antibody fragment of claim 75, where in said antibody fragment is chimeric.

79. The isolated antibody fragment of claim 75, wherein said antibody fragment is an antagonist of the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853.

80. The isolated antibody fragment of claim 75, wherein said antibody fragment is an agonist of the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853.

81. A composition comprising the isolated antibody fragment of claim 75, and a carrier.

82. A method of producing the isolated antibody fragment of claim 75, comprising:

(a) introducing an immunogen into an animal; and

(b) recovering said antibody fragment.

83. A method of detecting the polypeptide encoded by the human cDNA in ATCC Deposit No. 97853 in a biological sample comprising:

(a) contacting a biological sample with the isolated antibody fragment of claim 75; and

(b) determining the presence or absence of said polypeptide in said biological sample.--

Remarks

Support for the Amendments

The specification has been amended to correct inadvertent errors and to comply with formalities. Support for the amendments to the specification is found throughout the specification as filed. More particularly, the specification has been amended to reposition and amend the

reference to previous applications, to reflect the designation of Figures 1, 2, and 4 as Figures 1A and 1B, 2A and 2B, and 4A and 4B, to correct formalities in section headings, to reflect the new address of the American Type Culture Collection, to comply with 37 C.F.R. § 1.821(b), and to correct typographical and clerical errors. A substitute Sequence Listing is hereby submitted to correct a clerical error.

The heading: "Brief Description of the Figures" has been changed to "Brief Description of the Drawings."

All references to Figure 1 in the specification have been amended to recite Figure 1A and/or Figure 1B, as appropriate. Similarly, reference to Figure 2 has been changed to Figure 2A and 2B and reference to Figure 4 has been changed to Figure 4A and 4B.

Reference to sequence identifiers has been added to the brief description of Figures 1 and 2 on page 5 of the specification.

With respect to the correction of the NaCl and sodium citrate concentrations on page 11, lines 27-28 of the specification, Applicants submit that 5x SSC is a well-known solution used in hybridization solutions. SSC is normally made as a 20x stock solution, and then diluted accordingly for a particular use. The 20x SSC stock solution contains 3 M NaCl and 0.3 M trisodium citrate. *See, e.g., Gibco BRL Products and Reference Guide, 2000-2001* at page 22-24 (Exhibit A). To make a 5x SSC solution, the 20x solution must be diluted by one-fourth. Therefore, a 5x SSC solution contains 750 mM NaCl (3 M ÷ 4 = 750 mM) and 75 mM trisodium citrate (0.3 M ÷ 4 = 75 mM). One skilled in the art would have immediately recognized that the amount of ingredients listed in the specification for a 5x SSC solution was incorrect. Rather than describing a 5x SSC solution, made up of 750 mM NaCl and 75 mM trisodium citrate, the specification inaccurately listed the ingredient amounts for a 1x solution. The skilled artisan, in

recognizing the typographical error, could have easily adjusted the amount of ingredients described in the specification to properly make a 5x SSC solution.

On page 11, line 29, Applicants have noted a typographical error in the amount of salmon sperm DNA. The inclusion of agents such as salmon sperm DNA as blocking agents is well known in the art. *See, e.g.,* Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., (1997) at page 2.10.7 (Exhibit B). One skilled in the art would know that salmon sperm DNA is present in hybridization solutions in μg/ml quantities and thus would immediately recognize the above-described typographical error in the specification. *See id.* Further, the skilled artisan, in recognizing the typographical error, could easily have adjusted the amount of ingredients described in the specification to properly included 20 μg/ml denatured, sheared salmon sperm DNA in the hybridization solution.

On page 11, line 30, a degree sign which was inadvertently omitted has been added.

On page 22, lines 3, 6, 21, and 24, Applicants noted a clerical error in the designation of a conserved cysteine residue. The paragraph beginning on page 21, line 34, and continuing to page 22, line 9, refers, *inter alia*, to "the mature form(s) of a secreted protein . . . ." *See* specification at page 21, line 35. Applicants inadvertently used the numbering of the mature amino acid sequence rather than that used in SEQ ID NO:2. Therefore, the conserved cysteine residue referred to in this paragraph is in position 109 of the *mature* amino acid sequence, but the actual position of the conserved cysteine residue in the *full-length* amino acid sequence (*i.e.*, SEQ ID NO:2) is at position 132. Reference to SEQ ID NO:2 will show that the amino acid residue at position 109 is alanine, not cysteine, and in fact, the first cysteine residue in the polypeptide is at position 132. Applicants submit that one of skill in the art would realize this clerical error and understand that the conserved cysteine residue indeed is at position 132. For example, on page

22, lines 5-9, the reader is referred to Figure 2 to show that the cysteine residue is conserved among the four members of the TNF receptor family shown in the alignment. Figure 2A clearly indicates that the first cysteine which is conserved among all four sequences corresponds to residue 132 in DR4 (SEQ ID NO:2). *See* Figure 2A, lines 13-16 (SEQ ID NO:2 shown on line 16). The skilled artisan, in realizing that amino acid residue 109 is not cysteine, would immediately refer to Figure 2 for guidance as to the position of the conserved cysteine residue, and would realize that the conserved cysteine is actually residue 132.

Corrections of typographical errors on page 12, line 13, page 30, lines 4 and 37, and on page 39, line 29 are self-explanatory.

On page 33, line 7, there is a typographical error in the name of a herpes simplex virus gene. Support that the gene is actually designated "ICP34.5" is may be found in the attached abstract of Chou and Roizman, *J. Virol. 57*:629-637 (1986) (Exhibit C), the reference which originally identified ICP34.5 in herpes simplex virus.

On page 34, line 36, there is a typographical error in the name of the TNF-family ligand "lymphotoxin-α." Support that the ligand is actually designated "lymphotoxin-α," rather than lymphdtoxin, may be found in the attached abstract of Mauri, *et al.*, *Immunity* 8:21-30 (1998) (Exhibit D).

On page 37, line 34, there is a typographical error in the name of the bacterium *E. coli*. Applicants submit that it would be readily apparent to one of skill in the art what bacterial species was intended by Applicants.

Applicants assert that no new matter will be added to the specification if these formalities, clerical errors, and typographical errors are corrected, and respectfully request that the amendments to the specification be entered.

Support for the added claims 22 to 83 may be found throughout the specification, for example, at p. 8, lines 26-27; p. 8, line 35; p. 9, lines 5-16; p. 10, lines 22-33; p. 11, lines 1-15; p. 20, lines 20-24; p. 27, lines 22-32, p. 28, lines 1-21; p. 28, lines 30-32; p. 29, lines 16-24; p. 32, line 35 to p. 33, line 2; p. 34, lines 10-21; p. 36, lines 12-16, and in Figure 3.

Support for the polypeptide fragments of claims 25 and 47 can be found, *e.g.*, in the specification at page 10, lines 15-19; page 21, line 34 to page 23, line 27; and in SEQ ID NO:1 at pages 48 and 49. Specifically, it is noted at page 22, lines 2-4 that polypeptides with N-terminal amino acid deletions up to the cysteine-132 residue (as amended) may retain some biological activity. The specification at page 22, lines 27-28, in discussing these polypeptides with N-terminal deletions, notes that "polynucleotides encoding these polypeptides also are provided." Similarly, it is noted at page 22, lines 34-35, that polypeptides with C-terminal deletions up to the cysteine-221 residue may retain some biological activity. The specification at page 23, lines 18-19, in discussing these polypeptides with C-terminal deletions, notes that "polynucleotides encoding these polypeptides also are provided." Finally, it is noted at page 23, lines 20-23, that a polypeptide of the present invention may have both the above-noted N-terminal and C-terminal deletions. Therefore, a person of ordinary skill would have understood the present inventors to have been in possession of the claimed subject matter.

Applicants assert that the foregoing claim amendments do not add new matter.

The Sequence Listing

In compliance with 37 C.F.R. § 1.825(a), Applicants submit substitute sheets to amend the paper copy of the Sequence Listing as well as a substitute computer readable copy of the Sequence Listing. Applicants' Attorney hereby states that the changes made in the sequence listing do not include new matter.

In accordance with 37 C.F.R. § 1.825(b), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith are the same.

Applicants have discovered that there was an inadvertent error in entering sequences into the Sequence Listing, which resulted in the mis-identification of the oligonucleotide sequences disclosed on pages 38 through 42. The oligonucleotide sequence on page 38, line 6, was inadvertently omitted from the sequence listing, therefore, the remaining three oligonucleotide sequences were misnumbered in the sequence listing. To correct this error, applicants submit herewith a substitute Sequence Listing, in which the oligonucleotide sequence disclosed on page 38, line 6, which was originally listed in the specification as SEQ ID NO:9 has been added as SEQ ID NO:12. Accordingly, the oligonucleotide sequence disclosed on page 41, lines 20-21 and on page 42, line 20, which was originally identified in the specification as SEQ ID NO:10, is actually SEQ ID NO:9, the oligonucleotide sequence disclosed on page 41, lines 22-23, which was originally identified in the specification as SEQ ID NO:11, is actually SEQ ID NO:10, and the oligonucleotide sequence disclosed on page 42, line 28, which was originally identified in the specification as SEQ ID NO:12, is actually SEQ ID NO:11. Appropriate amendments have been made to the specification to conform to the substitute sequence listing. Since all of these oligonucleotides were disclosed in their entireties in the specification, this amendment adds no new matter.

Summary

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is respectfully requested

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

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Death Domain Containing Receptor 4

Field of the Invention

The present invention relates to a novel member of the tumor necrosis factor family of receptors. More specifically, isolated nucleic acid molecules are provided encoding human Death Domain Containing Receptor 4, sometimes herein "DR4". DR4 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR4 activity.

This application claims benefit of 35 U.S.C. section 119(e) based on copending U.S. Provisional Application Serial Nos. 60/035,722, filed January 28, 1997 and 60/037,829, filed February 5, 1997, both of which are incorporated herein by reference.

Background of the Invention

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF-α, lymphotoxin-α (LT- α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., *Biologicals,* 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., *supra*).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., *et al., Nature 356*:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. *et al., Science 259*:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. *et al., Cell 69*:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., *Science 264*:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (p55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia *et al., Cell 74*:845 (1993)).

Apoptosis, or programmed cell death, is a physiologic process essential to the normal development and homeostasis of multicellular organisms (H. Steller, *Science* 267, 1445-1449 (1995)). Derangements of apoptosis contribute to the pathogenesis of several human diseases including cancer, neurodegenerative disorders, and acquired immune deficiency syndrome (C.B. Thompson, *Science* 267, 1456-1462 (1995)). Recently, much attention has focused on the signal transduction and biological function of two cell surface death receptors, Fas/APO-1 and TNFR-1 (J.L. Cleveland, *et al., Cell* 81, 479-482 (1995); A. Fraser, *et al., Cell* 85, 781-784 (1996); S. Nagata, *et al., Science* 267, 1449-56 (1995)). Both are members of the TNF receptor family which also include TNFR-2, low affinity NGFR, CD40, and CD30, among others (C.A. Smith, *et al., Science 248*, 1019-23 (1990); M. Tewari, *et al.,* in *Modular Texts in Molecular and Cell Biology* M. Purton, Heldin, Carl, Ed. (Chapman and Hall, London, 1995). While family

members are defined by the presence of cysteine-rich repeats in their extracellular domains, Fas/APO-1 and TNFR-1 also share a region of intracellular homology, appropriately designated the "death domain", which is distantly related to the Drosophila suicide gene, reaper (P. Golstein, *et al., Cell 81*, 185-6 (1995); K. White *et al., Science* 264, 677-83 (1994)). This shared death domain suggests that both receptors interact with a related set of signal transducing molecules that, until recently, remained unidentified. Activation of Fas/APO-1 recruits the death domain-containing adapter molecule FADD/MORT1 (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M. P. Boldin, *et al., J. Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)), which in turn binds and presumably activates FLICE/MACH1, a member of the ICE/CED-3 family of pro-apoptotic proteases (M. Muzio *et al., Cell 85*, 817-827 (1996); M.P. Boldin, *et al., Cell 85*, 803-815 (1996)). While the central role of Fas/APO-1 is to trigger cell death, TNFR-1 can signal an array of diverse biological activities-many of which stem from its ability to activate NF-kB (L.A. Tartaglia, *et al., Immunol Today 13*, 151-3 (1992)). Accordingly, TNFR-1 recruits the multivalent adapter molecule TRADD, which like FADD, also contains a death domain (H. Hsu, *et al., Cell 81*, 495-504 (1995); H. Hsu, *et al., Cell 84*, 299-308 (1996)). Through its associations with a number of signaling molecules including FADD, TRAF2, and RIP, TRADD can signal both apoptosis and NF-kB activation (H. Hsu, *et al., Cell 84*, 299-308 (1996); H. Hsu, *et al., Immunity 4*, 387-396 (1996)).

Recently a new apoptosis inducing ligand was discovered. Wiley, S.R. et al., refer to the new molecule as TNF-related apoptosis-inducing ligand or ("TRAIL") (Immunity 3:673-682 (1995)). Pitti, R.M. et al., refer to the new molecule as Apo-2 ligand or ("Apo-2L"). This molecule was also disclosed in copending US Provisional Patent Application Serial No. 60/013405. For convenience, it will be referred to herein as TRAIL.

Unlike FAS ligand whose transcripts appear to be largely restricted to stimulated T-cells, significant levels of TRAIL are seen in many tissues, and it is constitutively transcribed by some cell lines. It has been shown that TRAIL acts independently from FAS ligand (Wiley, S.R., et al. (1995)), supra). Studies by Marsters, S.A. et al., have indicated that TRAIL activates apoptosis rapidly, within a time frame that is similar to death signalling by FAS/Apo-1L but much faster than TNF-induced apoptosis (Current Biology, 6:750-752 (1996)). All work to date suggest that the receptor for TRAIL is not one of the many known TNF-receptors.

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence

biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize the receptor for the newly discovered TRAIL ligand.

Summary of the Invention

The present invention provides for isolated nucleic acid molecules comprising nucleic acid sequences encoding the amino acid sequence shown in FIG. 1 (SEQ ID NO:2) or the amino acid sequence encoding the cDNA clone deposited as ATCC Deposit No. 97853 on January 21, 1997.

The present invention also provides vectors and host cells for recombinant expression of the nucleic acid molecules described herein, as well as to methods of making such vectors and host cells and for using them for production of DR4 polypeptides or peptides by recombinant techniques.

The invention further provides an isolated DR4 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention also provides diagnostic assays such as quantitative and diagnostic assays for detecting levels of DR4 protein. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of DR4, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors.

Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes. Cellular response to TNF-family ligands include not only normal physiological responses, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death-is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes. Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers, autoimmune disorders, viral infections, inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia and anorexia.

Thus, the invention further provides a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR4 polypeptide an effective amount of an agonist capable of increasing DR4 mediated signaling. Preferably, DR4 mediated signaling is increased to treat a disease wherein decreased apoptosis is exhibited.

In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR4 polypeptide an effective amount of an antagonist capable of decreasing DR4 mediated signaling. Preferably, DR4 mediated signaling is decreased to treat a disease wherein increased apoptosis is exhibited.

Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below. Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DR4 polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By the invention, a cell expressing the DR4 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

Brief Description of the Figures

FIG. 1 shows the nucleotide and deduced amino acid sequence of DR4. It is predicted that amino acids 1-23 constitute the signal peptide, amino acids 24-238 constitute the extracellular domain, amino acids 239-264 constitute the transmembrane domain, and amino acids 265-468 constitute the intracellular domain of which amino acids 379-422 constitute the death domain.

FIG. 2 shows the regions of similarity between the amino acid sequences of DR4, human tumor necrosis factor receptor 1 (SEQ ID NO:3), human Fas protein (SEQ ID NO:4), and the death domain containing receptor 3 (DR3) (SEQ ID NO:5).

FIG. 3 shows an analysis of the DR4 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues 35-92, 114-160, 169-240, 267-298, 330-364, 391-404, and 418-465 in Figure 1 correspond to the shown highly antigenic regions of the DR4 protein.

FIG. 4 shows the nucleotide sequences of related nucleic acid fragments HTOIY07R (SEQ ID NO:6) and HTXEY80R (SEQ ID NO:7).

FIG. 5A and 5B show the ability of DR4 to induce apoptosis in the cell lines MCF7 and 293. FIG.5C shows the ability of death protease inhibitors z-VAD-fmk and CrmA to inhibit the apoptotic action of DR4.

FIG. 6A shows the ability of a soluble extracellular DR4-Fc fusion to block the apoptotic inducing ability of TRAIL. FIG. 6B shows the inability of soluble extracellular DR4-Fc fusion to block the apoptotic inducing ability of TNF-alpha.

Detailed Description of the Preferred Embodiments

The present invention provides isolated nucleic acid molecules comprising a nucleic acid sequence encoding the DR4 polypeptide whose amino acid sequence is shown in FIG. 1 (SEQ ID NO:2), or a fragment of the polypeptide. The DR4 polypeptide of the present invention shares sequence homology with human TNFR-I, DR3 and Fas ligand (FIG. 2). The nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) was obtained by sequencing cDNA clones such as HCUDS60, which was deposited on January 21, 1997 at the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, and given Accession Number 97853. The deposited clone is contained in the pBK plasmid (Stratagene, LaJolla, CA).

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be

completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "isolated" polypeptide or protein is intended a polypeptide or protein removed from its native environment. For example, recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purposed of the invention as are native or recombinant polypeptides which have been substantially purified by any suitable technique such as, for example, the single-step purification method disclosed in Smith and Johnson, *Gene 67*:31-40 (1988).

Using the information provided herein, such as the nucleic acid sequence set out in FIG. 1, a nucleic acid molecule of the present invention encoding a DR4 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the gene of the present invention has also been identified in cDNA libraries of the following tissues: amniotic cells, heart, liver cancer, kidney, leukocyte, activated T-cell, K562 plus PMA, W138 cells, Th2 cells, human tonsils, and CD34 depleted buffy coat (cord blood).

The DR4 gene contains an open reading frame encoding a mature protein of about 445 amino acid residues whose initiation codon is at position 19-21 of the nucleotide sequence shown in FIG. 1 (SEQ ID NO.1), with a leader sequence of about 23 amino acid residues (i.e., a total protein length of 468 amino acids), and a deduced molecular weight of about 50 kDa. Of known members of the TNF receptor family, the DR4 polypeptide of the invention shares the greatest degree of homology with human TNFRl and DR3 polypeptides shown in Fig. 2, including significant sequence homology over the multiple Cysteine Rich domains.

In addition to the sequence homology exhibited between DR4 and other death domain containing receptors, DR4 has been shown to bind to TRAIL and to induce apoptosis when transiently expressed. MCF7 human breast carcinoma cells and 293 cells were transiently transfected with a DR4 expressing construct, as described in Example 5. As shown in Figures 5A and 5B a substantial proportion of transfected cells underwent the morphological changes characteristic of apoptosis. As anticipated, deletion of the death domain abolished the ability of DR4 to engage the death pathway. As can be seen in Figure 5C, DR4-induced apoptosis was efficiently blocked by inhibitors of death proteases including z-VAD-fmk, an irreversible broad spectrum caspase inhibitor and CrmA, a cowpox virus encoded serpin that preferentially inhibits apical caspases such as FLICE/MACH-1 (caspase-8). Since TNFR-1, CD-95 and DR3-induced apoptosis is also attenuated by these same inhibitors, it is likely that the downstream death effector molecules are similar in nature.

To determine if DR4 was capable of binding TRAIL, the extracellular ligand binding domain of DR4 was expressed as a fusion to the Fc region of human IgG (DR4-Fc). TRAIL selectively bound to DR4-Fc but not to corresponding extracellular domains of TNFR-1 or CD-95, also expressed as Fc fusions, data not shown. Additionally, DR4-Fc did not bind either TNF alpha or Fas ligand under conditions where both of these ligands bound their cognate receptors.

The ability of TRAIL to induce apoptosis in MCF7 cells was specifically blocked by DR4-Fc but not influenced by TNFR1-Fc, CD95-Fc or Fc alone (Figure 6A). Further, as expected, TNF alpha-induced apoptosis was inhibited by TNFR-1-Fc but not by DR4-Fc, CD95-Fc or Fc alone (Figure 6B).

Taken together, the data described above indicate that DR4 is a death domain containing receptor with the ability to induce apoptosis and is a receptor for TRAIL a known apoptosis inducing ligand.

As indicated, the present invention also provides the mature form(s) of the DR4 protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit No. 97853, and as shown in Figure 1 (SEQ ID NO:2). By the mature DR4 protein having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit No. 97853, is meant the mature form(s) of the DR4 protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature DR4 having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853, may or may not differ from the predicted "mature" DR4 protein shown in Figure 1 (amino acids from about 24 to about 468) depending on the accuracy of the predicted cleavage site based on computer analysis.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (*Virus Res.* *3*:271-286 (1985)) and von Heinje (*Nucleic Acids Res.*

14:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the predicted amino acid sequence of the complete DR4 polypeptide of the present invention was analyzed by a computer program ("PSORT"). (*see* K. Nakai and M. Kanehisa, *Genomics 14*:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 23 and 24 in Figure 1 (SEQ ID NO:2). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heine. von Heinje, *supra*. Thus, the leader sequence for the DR4 protein is predicted to consist of amino acid residues 1-23, underlined in Figure 1 (SEQ ID NO:2), while the predicted mature DR4 protein consists of residues 24-468.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DR4 DNA molecules comprising an open reading frame (ORF) shown in FIG. 1 (SEQ ID NO:1) and further include DNA molecules which comprise a sequence substantially different than all or part of the ORF whose initiation codon is at position 19-21 of the nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) but which, due to the degeneracy of the genetic code, still encode the DR4 polypeptide or a fragment thereof. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the DR4 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97853 on January 21, 1997. Preferably, these nucleic acid molecules will encode the mature polypeptide encoded by the above-described deposited cDNA clone. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) or the nucleotide sequence of the DR4 cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated DNA molecules and fragments thereof are useful as DNA probes for gene mapping by *in situ* hybridization of the DR4 gene in human tissue by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By fragments of an isolated DNA molecule having the nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) are intended DNA fragments at least 20 bp, and more preferably at least 30 bp in length which are useful as DNA probes as discussed above. of course larger DNA fragments 50-1500 bp in length are also useful as DNA probes according to the present invention as are DNA fragments corresponding to most, if not all, of the nucleotide sequence shown in FIG. 1 (SEQ ID NO:1). By a fragment at least 20 bp in length, for example, is intended fragments which include 20 or more bases from the nucleotide sequence in FIG. 1 (SEQ ID NO:1).

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the DR4 extracellular domain (amino acid residues from about 24 to about 238 in FIG. 1 (SEQ ID NO:2)); a polypeptide comprising the DR4 transmembrane domain (amino acid residues from about 239 to about 264 in FIG. 1 (SEQ ID NO:2)); a polypeptide comprising the DR4 intracellular domain (amino acid residues from about 265 to about 468 in FIG. 1 (SEQ ID NO:2)); and a polypeptide comprising the DR4 death domain (amino acid residues from about 379 to about 422 in FIG. 1 (SEQ ID NO:2)). Since the location of these domains have been predicted by computer graphics, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 residues) depending on the criteria used to define the domain.

Preferred nucleic acid fragments of the invention encode a full-length DR4 polypeptide lacking the nucleotides encoding the amino-terminal methionine (nucleotides 19-21 in SEQ ID NO:1) as it is known that the methionine is cleaved naturally and such sequences maybe useful in genetically engineering DR4 expression vectors. Polypeptides encoded by such polynucleotides are also contemplated by the invention.

Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding epitope-bearing portions of the DR4 protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 35 to about 92 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 114 to about 160 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 169 to about 240 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 267 to about 298 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 330 to about 364 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 391 to about 404 in Figure 1 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 418 to about 465 in Figure 1 (SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the DR4 protein. Methods for determining other such epitope-bearing portions of the DR4 protein are described in detail below.

In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 as follows: HTOIY07R (SEQ ID NO:6) and HTXEY80R (SEQ ID NO:7) both shown in Fig. 4.

Further, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:1 from residue 365 to 1,424.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC Deposit No. 97853. By "stringent hybridization conditions" is intended overnight incubation at 42 C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the

reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1) or Figure 2 (SEQ ID NO:3).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3 terminal poly(A) tract of the DR4 cDNA shown in Figure 1 (SEQ ID NO:1)), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode the DR4 polypeptide may include, but are not limited to the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as those encoding a leader or secretary sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing - including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag corresponds to an epitope derived of influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*:767 (1984), for instance.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode for fragments, analogs or derivatives of the DR4 polypeptide. Variants may occur naturally, such as an allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions which may involve one or more nucleotides. The variants may be

altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Further embodiments of the invention include isolated nucleic acid molecules that are at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to (a) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence; (b) nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence but lacking the amino terminal methionine; (c) a nucleotide sequence encoding the mature DR4 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 24 to about 468 in Figure 1 (SEQ ID NO:2); (d) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97853; (e) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader but lacking the amino terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853; (f) a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853; (g) a nucleotide sequence that encodes the DR4 extracellular domain, (h) a nucleotide sequence that encodes the DR4 transmembrane domain, (i) a nucleotide sequence that encodes the DR4 intracellular domain, (j) a nucleotide sequence that encodes the DR4 death domain; or (k) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a DR4 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the DR4 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions,

interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1 or to the nucleotide sequences of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having DR4 activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having DR4 activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having DR4 activity include, *inter alia,* (1) isolating the DR4 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the DR4 gene, as described in Verma *et al., Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting DR4 mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs which do, in fact, encode a polypeptide having DR4 protein activity. By "a polypeptide having DR4 activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the DR4 protein of the invention (either the full-length protein or, preferably, the mature protein), as measured in a particular biological assay. For example, DR4 protein activity can be

measured using the cell death assays performed essentially as previously described (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995); A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)) or as set forth in Example 5, below. In MCF7 cells, plasmids encoding full-length DR4 or a candidate death domain containing receptors are co-transfected with the pLantern reporter construct encoding green fluorescent protein. Nuclei of cells transfected with DR4 will exhibit apoptotic morphology as assessed by DAPI staining. Similar to TNFR-1 and Fas/APO-1 (M. Muzio, *et al., Cell 85*, 817-827 (1996); M. P. Boldin, *et al., Cell 85*, 803-815 (1996); M. Tewari, *et al., J Biol Chem 270*, 3255-60 (1995)), DR4-induced apoptosis is blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) will encode a polypeptide "having DR4 protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having DR4 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J.U. *et al.,* "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Polynucleotide assays

This invention is also related to the use of the DR4 polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a mutated form of DR4 associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to a disease which results from under-expression over-expression or altered expression of DR4 or a soluble form thereof, such as, for example, tumors or autoimmune disease.

Individuals carrying mutations in the DR4 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. (Saiki *et al., Nature 324*:163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding DR4 can be used to identify and analyze DR4 expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled DR4 RNA or alternatively, radiolabeled DR4 antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al., Science 230*:1242 (1985)).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and Sl protection or the chemical cleavage method (e.g., Cotton *et al., Proc. Natl. Acad. Sci. USA 85*: 4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

Chromosome assays

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a DR4 gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA the is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60. For a review of this technique, see Verma *et al., Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes)).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Vectors and Host Cells

The present invention also relates to vectors which include DNA molecules of the present invention, host cells which are genetically engineered with vectors of

the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells can be genetically engineered to incorporate nucleic acid molecules and express polypeptides of the present invention. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s)), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the *E. coli lac*, *trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name just a few of the well-known promoters. In general, expression constructs will contain sites for transcription, initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the

constructs will include a translation initiating AUG at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, such regions will operate by controlling transcription, such as repressor binding sites and enhancers, among others.

Vectors for propagation and expression generally will include selectable markers. Such markers also may be suitable for amplification or the vectors may contain additional markers for this purpose. In this regard, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells. Preferred markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing *E. coli* and other bacteria.

The vector containing the appropriate DNA sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well known techniques suitable to expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as *E. coli*, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Hosts for of a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure readily to select a host for expressing a polypeptides in accordance with this aspect of the present invention.

Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene: and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors available to those of skill in the art.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

The present invention also relates to host cells containing the above-described constructs discussed above. The host cell can be a higher eukaryotic cell,

such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al., Basic Methods in Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5- has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry*, Vol. 270, No. 16:9459-9471 (1995).

The DR4 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography

("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

DR4 polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of DR4. Among these are applications in treatment of tumors, resistance to parasites, bacteria and viruses, to induce proliferation of T-cells, endothelial cells and certain hematopoietic cells, to treat restenosis, graft vs. host disease, to regulate anti-viral responses and to prevent certain autoimmune diseases after stimulation of DR4 by an agonist. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are discussed further below.

DR4 Polypeptides and Fragments

The invention further provides an isolated DR4 polypeptide having the amino acid sequence shown in FIG. 1 [SEQ ID NO:2] or a peptide or polypeptide comprising a portion of the above polypeptides.

To improve or alter the characteristics of DR4 polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., J. Biol. Chem., 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues

were missing. In the present case, since the protein of the invention is a member of the death domain containing receptor (DDCR) polypeptide family, deletions of N-terminal amino acids up to the cysteine residue at position 109 in SEQ ID NO:2 may retain some biological activity such as the ability to induce apoptosis. Polypeptides having further N-terminal deletions including the cysteine residue at position 109 (C-109) in SEQ ID NO:2 would not be expected to retain such biological activities because this residue is conserved among family members, see Figure 2, may be required for forming a disulfide bridge to provide structural stability which is needed for receptor binding.

However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or extracellular domain of the protein generally will be retained when less than the majority of the residues of the complete or extracellular domain protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of DR4 shown in SEQ ID NO:2, up to C-109 residue, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n-468 of SEQ ID NO:2, where n is an integer in the range of 1-109 where C-109 is the first residue from the N-terminus of the extracellular domain of the DR4 polypeptide (shown in SEQ ID NO:2) believed to be required for receptor-ligand binding (e.g., TRAIL binding) activity of the DR4 protein. Polynucleotides encoding these polypeptides also are provided.

Similarly, many examples of biologically functional C-terminal deletion muteins are known. For instance, interferon gamma shows up to ten times higher activities by deleting 8-10 amino acid residues from the carboxy terminus of the protein (Döbeli et al., J. Biotechnology 7:199-216 (1988). In the present case, since the protein of the invention is a member of the DDCR polypeptide family, deletions of C-terminal amino acids up to the cysteine at position 221 (C-221) of SEQ ID NO:2 may retain some biological activity such receptor binding. Polypeptides having further C-terminal deletions including C-221 of SEQ ID NO:2 would not be expected to retain such biological activities because this residue is conserved among DDCR family members and is required for forming a disulfide bridge to provide structural stability which is needed for receptor-ligand binding.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or extracellular domain of the protein generally will be retained when less than the majority of the residues of the complete or extracellular domain are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of the DR4 shown in SEQ ID NO:2, up to C-221 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m of the amino acid sequence in SEQ ID NO:2, where m is any integer in the range of 221-468 and residue C-221 is the position of the first residue from the C- terminus of the complete DR4 polypeptide (shown in SEQ ID NO:2) believed to be required for receptor binding activity of the DR4 protein. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues n-m of SEQ ID NO:2, where n and m are integers as described above.

Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of the complete DR4 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853, where this portion excludes from 1 to about 108 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853, or from 1 to about 247 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

Preferred amongst the N- and C-terminal deletion mutants are those comprising only a portion of the extracellular domain; i.e., within residues 24-238, since any portion therein is expected to be soluble.

It will be recognized in the art that some amino acid sequence of DR4 can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be

critical areas on the protein which determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains.

Thus, the invention further includes variations of the DR4 protein which show substantial DR4 protein activity or which include regions of DR4 such as the protein fragments discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U. *et al., Science 247:*1306-1310 (1990).

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the DR4 protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol.* 2:331-340 (1967); Robbins *et al., Diabetes 36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the DR4 receptor of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the DR4 protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* *244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced

version of the DR4 polypeptide is substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention also include the polypeptide encoded by the deposited cDNA including the leader, the mature polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 1 (SEQ ID NO:2) including the leader, the polypeptide of Figure 1 (SEQ ID NO:2) minus the amino terminal methionine, the polypeptide of Figure 1 (SEQ ID NO:2) minus the leader, the extracellular domain, the transmembrane domain, the intracellular domain, the death domain, soluble polypeptides comprising all or part of the extracellular and intracelluar domains but lacking the transmembrane domain as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA clones, to the polypeptide of Figure 1 (SEQ ID NO:2) and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a DR4 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the DR4 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figure 1 (SEQ ID NO:2) or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters

are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The present inventors have discovered that the DR4 polypeptide is a 468 residue protein exhibiting three main structural domains. First, the ligand binding domain was identified within residues from about 24 to about 238 in FIG. 1 [SEQ ID NO:2]. Second, the transmembrane domain was identified within residues from about 239 to about 264 in FIG. 1 [SEQ ID NO:2]. Third, the intracellular domain was identified within residues from about 265 to about 468 in FIG. 1 [SEQ ID NO:2]. Importantly, the intracellular domain includes a death domain at residues from about 379 to about 422. Further preferred fragments of the polypeptide shown in FIG. 1 [SEQ ID NO:2] include the mature protein from residues about 24 to about 468 and soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain.

The invention further provides DR4 polypeptides encoded by the deposited cDNA clone including the leader and DR4 polypeptide fragments selected from the mature protein, the extracellular domain, the transmembrane domain, the intracellular domain, and the death domain.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide described herein. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science 219:*660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate DR4-specific antibodies include: a polypeptide comprising amino acid residues from about 35 to about 92 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 114 to about 160 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 169 to about 240 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 267 to about 298 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 330 to about 364 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 391 to about 404 in Figure 1 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 418 to about 465 in Figure 1 (SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the DR4 protein.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R.A., "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids," *Proc. Natl. Acad. Sci. USA 82*:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

As one of skill in the art will appreciate, DR4 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al., Nature 331*:84- 86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the

monomeric DR4 protein or protein fragment alone (Fountoulakis *et al., J Biochem* *270*:3958-3964 (1995)).

Polypeptide assays

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of DR4 protein, or the soluble form thereof, in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of DR4, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors, for example. Assay techniques that can be used to determine levels of a protein, such as a DR4 protein of the present invention, or a soluble form thereof, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Assaying DR4 protein levels in a biological sample can occur using any art-known method. Preferred for assaying DR4 protein levels in a biological sample are antibody-based techniques. For example, DR4 protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell. Biol. 105:*3087-3096 (1987)).

Other antibody-based methods useful for detecting DR4 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable labels are known in the art and include enzyme labels, such as glucose oxidase, radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulphur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Therapeutics

The Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (Goeddel, D.V. *et al.*, "Tumor Necrosis Factors: Gene Structure and Biological Activities," *Symp. Quant. Biol. 51*:597-609 (1986), Cold Spring Harbor; Beutler, B., and Cerami, A., *Annu. Rev. Biochem. 57*:505-518 (1988); Old, L.J., *Sci. Am. 258*:59-75 (1988); Fiers, W., *FEBS Lett. 285*:199-224 (1991)). The TNF-family ligands induce such various cellular responses by binding to TNF-family receptors, including the DR4 of the

present invention. Cells which express the DR4 polypeptide and are believed to have a potent cellular response to DR4 ligands include amniotic cells, heart, liver cancer, kidney, peripheral blood leukocytes, activated T-cells, tissue corresponding to Th2 cells, human tonsils, and CD34 depleted buffy coat (cord blood).. By "a cellular response to a TNF-family ligand" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death-is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes (Ameisen, J.C., *AXDS* *8*:1197-1213 (1994); Krammer, P.H. *et al., Curr. Opin. Immunol.* *6*:279-289 (1994)).

Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, such as breast cancer, prostrate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), information graft v. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Thus, in one aspect, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR4 polypeptide an effective amount of DR4 ligand, analog or an agonist capable of increasing DR4 mediated signaling. Preferably, DR4 mediated signaling is increased to treat a disease wherein decreased apoptosis or decreased cytokine and adhesion molecule expression is exhibited. An agonist can include soluble forms of DR4 and monoclonal antibodies directed against the DR4 polypeptide.

In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the, DR4 polypeptide an effective amount of an antagonist capable of decreasing DR4 mediated signaling. Preferably, DR4 mediated signaling is decreased to treat a disease wherein increased apoptosis or NFkB expression is

exhibited. An antagonist can include soluble forms of DR4 and monoclonal antibodies directed against the DR4 polypeptide.

By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating apoptosis. By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below.

One such screening procedure involves the use of melanophores which are transfected to express the receptor of the present invention. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed, for example, for screening for a compound which inhibits (or enhances) activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both a TNF-family ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is an antagonist or agonist of the ligand/receptor signaling pathway.

Other screening techniques include the use of cells which express the receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science 246*:181-296 (October 1989). For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding the receptor into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing in cells a construct wherein the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase signal.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining

inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

Further screening assays for agonist and antagonist of the present invention are described in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992).

Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DR4 polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or a TNF-family ligand (e.g., determining or estimating an increase or decrease in T cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing the DR4 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

Agonist according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor , neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonist include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and -amyloid peptide. (*Science 267*:1457-1458 (1995)). Further preferred agonist include polyclonal and monoclonal antibodies raised against the DR4 polypeptide, or a fragment thereof. Such agonist antibodies raised against a TNF-family receptor are disclosed in Tartaglia, L.A., *et al., Proc. Natl. Acad. Sci. USA 88*:9292-9296 (1991); and Tartaglia, L.A., and Goeddel,

D.V., *J. Biol. Chem. 267* (7):4304-4307 (1992) See, also, PCT Application WO 94/09137.

Antagonist according to the present invention include naturally occurring and synthetic compounds such as, for example, the CD40 ligand, neutral amino acids, zinc, estrogen, androgens, viral genes (such as Adenovirus *ElB*, Baculovirus *p35* and *IAP*, Cowpox virus *crmA*, Epstein-Barr virus *BHRF1*, *LMP-1*, African swine fever virus *LMW5-HL*, and Herpesvirus yl 34.5), calpain inhibitors, cysteine protease inhibitors, and tumor promoters (such as PMA, Phenobarbital, and -Hexachlorocyclohexane).

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem. 56*:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee *et al., Nucleic Acids Research 6*:3073 (1979); Cooney *et al., Science 241*:456 (1988); and Dervan *et al., Science 251*:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the receptor.

Further antagonist according to the present invention include soluble forms of DR4, i.e.,DR4 fragments that include the ligand binding domain from the extracellular region of the full length receptor. Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize DR4 mediated signaling by competing with the cell surface DR4 for binding to TNF-family ligands. Thus, soluble forms of the receptor that include the ligand binding domain are novel cytokines capable of inhibiting apoptosis induced by TNF-family ligands. These are preferably expressed as dimers or trimers, since these have been shown to be superior to monomeric forms of soluble receptor as antagonists, e.g., IgGFc-TNF receptor family fusions. Other such cytokines are known in the art and include Fas B (a soluble form of the mouse Fas receptor) that acts physiologically to limit

apoptosis induced by Fas ligand (Hughes, D.P. and Crispe, I.N., *J. Exp. Med.* *182*:1395-1401 (1995)).

The experiments set forth in Example 5 demonstrates that DR4 is a death domain-containing molecule capable of triggering apoptosis which is important in the regulation of the immune system. In addition, the experiments set forth below demonstrate that DR4-induced apoptosis was blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk. Thus, inhibitors of ICE-like proteases, FADD-DN and FLICE-DN/MACHa1C360S could also be used as antagonists for DR4 activity.

The term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of binding an antigen. Fab and F $(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)).

Antibodies according to the present invention may be prepared by any of a variety of methods using DR4 immunogens of the present invention. As indicated, such DR4 immunogens include the full length DR4 polypeptide (which may or may not include the leader sequence) and DR4 polypeptide fragments such as the ligand binding domain, the transmembrane domain, the intracellular domain and the death domain.

Proteins and other compounds which bind the DR4 domains are also candidate agonist and antagonist according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature 340*:245-246 (1989)). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (Gyuris, J. *et al., Cell 75*:791-803 (1993); Zervos, A.S. *et al., Cell 72*:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to either the DR4 ligand binding domain or to the DR4 intracellular domain. Such compounds are good candidate agonist and antagonist of the present invention.

By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, DR4 ligands including TRAIL, TNF-α, lymphdtoxin-α (LT-α, also known as TNF-β), LT-β (found in complex

heterotrimer LT-α2-β), FasL, CD40, CD27, CD30, 4-IBB, OX40 and nerve growth factor (NGF).

Representative therapeutic applications of the present invention are discussed in-more detail below. The state of immunodeficiency that defines AIDS is secondary to a decrease in the number and function of $CD4^+$ T-lymphocytes. Recent reports estimate the daily loss of $CD4^+$ T cells to be between 3.5×10^7 and 2×10^9 cells (Wei X., *et al., Nature 373*:117-122 (1995)). One cause of $CD4^+$ T cell depletion in the setting of HIV infection is believed to be HIV-induced apoptosis. Indeed, HIV-induced apoptotic cell death has been demonstrated not only *in vitro* but also, more importantly, in infected individuals (Ameisen, J.C., *AIDS 8*:1197-1213 (1994) ; Finkel, T.H., and Banda, N.K., *Curr. Opin. Immunol. 6*:605-615(1995); Muro-Cacho, C.A. *et al., J. Immunol. 154*:5555-5566 (1995)). Furthermore, apoptosis and $CD4^+$ T-lymphocyte depletion is tightly correlated in different animal models of AIDS (Brunner, T., *et al., Nature 373*:441-444 (1995); Gougeon, M.L., *et al., AIDS Res. Hum. Retroviruses 9*:553-563 (1993)) and, apoptosis is not observed in those animal models in which viral replication does not result in AIDS (Gougeon, M.L. *et al., AIDS Res. Hum. Retroviruses 9*:553-563 (1993)). Further data indicates that uninfected but primed or activated T lymphocytes from HIV-infected individuals undergo apoptosis after encountering the TNF-family ligand FasL. Using monocytic cell lines that result in death following HIV infection, it has been demonstrated that infection of U937 cells with HIV results in the *de novo* expression of FasL and that FasL mediates HIV-induced apoptosis (Badley, A.D. *et al., J. Virol. 70*:199-206 (1996)). Further the TNF-family ligand was detectable in uninfected macrophages and its expression was upregulated following HIV infection resulting in selective killing of uninfected CD4 T-lymphocytes (Badley, A.D *et al., J. Virol. 70*:199-206 (1996)). Thus, by the invention, a method for treating HIV^+ individuals is provided which involves administering an antagonist of the present invention to reduce selective killing of CD4 T-lymphocytes. Modes of administration and dosages are discussed in detail below.

In rejection of an allograft, the immune system of the recipient animal has not previously been primed to respond because the immune system for the most part is only primed by environmental antigens. Tissues from other members of the same species have not been presented in the same way that, for example, viruses and bacteria have been presented. In the case of allograft rejection, immunosuppressive regimens are designed to prevent the immune system from reaching the effector stage. However, the immune profile of xenograft rejection may resemble disease recurrence more that allograft rejection. In the case of disease recurrence, the

immune system has already been activated, as evidenced by destruction of the native islet cells. Therefore, in disease recurrence the immune system is already at the effector stage. Agonist of the present invention are able to suppress the immune response to both allografts and xenografts because lymphocytes activated and differentiated into effector cells will express the DR4 polypeptide, and thereby are susceptible to compounds which enhance apoptosis. Thus, the present invention further provides a method for creating immune privileged tissues. Antagonist of the invention can further be used in the treatment of Inflammatory Bowel-Disease.

DR4 antagonists may be useful for treating inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

In addition, due to lymphoblast expression of DR4, soluble DR4, agonist or antagonist mABs may be used to treat this form of cancer. Further, soluble DR4 or neutralizing mABs may be used to treat various chronic and acute forms of inflammation such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

Modes of Administration

The agonist or antagonists described herein can be administered *in vitro, ex vivo*, or *in vivo* to cells which express the receptor of the present invention. By administration of an "effective amount" of an agonist or antagonist is intended an amount of the compound that is sufficient to enhance or inhibit a cellular response to a TNF-family ligand and include polypeptides. In particular, by administration of an "effective amount" of an agonist or antagonists is intended an amount effective to enhance or inhibit DR4 mediated apoptosis. Of course, where apoptosis is to be enhanced, an agonist according to the present invention can be co-administered with a TNF-family ligand. One of ordinary skill will appreciate that effective amounts of an agonist or antagonist can be determined empirically and may be employed in pure form or in pharmaceutically acceptable salt, ester or prodrug form. The agonist or antagonist may be administered in compositions in combination with one or more pharmaceutically acceptable excipients.

It will be understood that, when administered to a human patient, the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgement. The specific therapeutically effective dose level for any particular patient will depend upon factors well known in the medical arts.

As a general proposition, the total pharmaceutically effective amount of DR4 polypeptide administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this

will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the DR4 agonists or antagonists is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Dosaging may also be arranged in a patient specific manner to provide a predetermined concentration of an agonist or antagonist in the blood, as determined by the RIA technique. Thus patient dosaging may be adjusted to achieve regular on-going trough blood levels, as measured by RIA, on the order of from 50 to 1000 ng/ml, preferably 150 to 500 ng/ml.

Pharmaceutical compositions are provided comprising an agonist or antagonist and a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. Importantly, by co-administering an agonist and a TNF-family ligand, clinical side effects can be reduced by using lower doses of both the ligand and the agonist. It will be understood that the agonist can be "co-administered" either before, after, or simultaneously with the TNF-family ligand, depending on the exigencies of a particular therapeutic application. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Pharmaceutical compositions of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use.

In addition to soluble DR4 polypeptides, DR4 polypeptide containing the transmembrane region can also be used when appropriately solubilized by including detergents, such as CHAPS or NP-40, with buffer.

Example 1: Expression and Purification in E. coli

The DNA sequence encoding the mature DR4 protein in the deposited cDNA clone (ATCC No. 97853) is amplified using PCR oligonucleotide primers specific to the amino terminal sequences of the DR4 protein and to vector sequences 3' to

the gene. Additional nucleotides containing restriction sites to facilitate cloning are added to the 5' and 3' sequences respectively.

The following primers are used for expression of DR4 extracellular domain in *E. coli* 5' primer 5'-GCGGCATGCATGATCAATCAATTGGCAC-3' (SEQ ID NO:8) contains the underlined SphI site. 3' primer 5'-GCGAAGCTTTCAATTATGTCCATTGCCTG-3' (SEQ ID NO:9) contains the underlined HindIII site. Vector is pQE60.

The restriction sites are convenient to restriction enzyme sites in the bacterial expression vector pQE60, which are used for bacterial expression in these examples. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS").

The amplified DR4 DNA and the vector pQE60 both are digested with SphI and HindIII and the digested DNAs are then ligated together. Insertion of the DDCR protein DNA into the restricted pQE60 vector places the DR4 protein coding region downstream of and operably linked to the vector's IPTG-inducible promoter and in-frame with an initiating AUG appropriately positioned for translation of DR4 protein.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures. Such procedures are described in Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing DR4 protein, is available commercially from Qiagen.

Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml).

The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600nm ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from *lac* repressor sensitive promoters, by inactivating the *lac*I repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation and disrupted, by standard methods. Inclusion bodies are purified

from the disrupted cells using routine collection techniques, and protein is solubilized from the inclusion bodies into 8M urea. The 8M urea solution containing the solubilized protein is passed over a PD-10 column in 2X phosphate-buffered saline ("PBS"), thereby removing the urea, exchanging the buffer and refolding the protein. The protein is purified by a further step of chromatography to remove endotoxin. Then, it is sterile filtered. The sterile filtered protein preparation is stored in 2X PBS at a concentration of 95 μ/ml.

Example 2: Expression in Mammalian Cells

Most of the vectors used for the transient expression of a given gene sequence in mammalian cells carry the SV40 origin of replication. This allows the replication of the vector to high copy numbers in cells (e.g. COS cells) which express the T antigen required for the initiation of viral DNA synthesis. Any other mammalian cell line can also be utilized for this purpose.

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g. RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, also cellular signals can be used (e.g. human actin, promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC67109). Mammalian host cells that could be used include, human Hela, 283, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1 African green monkey cells, quail QC1-3 cells, mouse L cells and Chinese hamster ovary cells such as

Alternatively, a gene of interest can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) is a useful marker to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Using this marker, the mammalian cells are grown in increasing amounts of methotrexate for selection and the cells with the highest resistance are

selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

The expression vectors pCl and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al., Molecular and Cellular Biology* 438:44701 (March 1985)), plus a fragment of the CMV-enhancer (Boshart *et al., Cell 41*:521-530 (1985)). Multiple cloning sites, e.g. with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of DR4 polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J. Biol. Chem. 253*:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta, 1097*:107-143, Page, M. J. and Sydenham, M. A. 1991, *Biotechnology 9*:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed.. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, et al., *Molecular and Cellular Biology*, March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart et al., *Cell 41*:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, Xba I, and Asp718. Behind these cloning sites the plasmid

contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human ß-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the DR4 polypeptide in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA 89*:5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzyme BamHI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the desired portion of the gene. The 5' primer containing the underlined BamHI site, a Kozak sequence, and an AUG start codon, has the following sequence: 5' GCG<u>GGATCC</u>GCCATCATGGCGCCACCACCAGCTAGA 3' (SEQ ID NO:10). The 3' primer, containing the underlined BamHI site, has the following sequence: 5' GCG<u>GGATCC</u>TCACTCCAAGGACACGGCAGAGCC 3' (SEQ ID NO:11).

The amplified fragment is digested with the endonuclease BamHI and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1

mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 µM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 3

Cloning and expression of the soluble extracellular domain of DR4 in a baculovirus expression system

The cDNA sequence encoding the soluble extracellular domain of DR4 protein in the deposited clone (ATCC No. 97853) is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer for DR4 has the sequence 5' GCG<u>GGATCC</u>GCCATCATGGCGCCACCACCAGCTAGA 3' (SEQ ID NO:10) containing the underlined BamHI restriction enzyme site. Inserted into an expression vector, as described below, the 5' end of the amplified fragment encoding DR4 provides an efficient cleavage signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987) is appropriately located in the vector portion of the construct.

The 3' primer for both DR4 has the sequence 5' GCG<u>GGATCC</u>TCAATTATGTCCATTGCCTG 3' (SEQ ID NO:12) containing the underlined BamHI restriction followed by nucleotides complementary to the DR4 nucleotide sequence set out in FIG. 1, followed by the stop codon.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean, " BIO 101 Inc., La Jolla, Ca.) The fragment then is digested with BamHI and Asp718 and again is purified on a 1% agarose gel.

The vector pA2 is used to express the DR4 protein in the baculovirus expression system, using standard methods, such as those described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

This expression vector contains the strong polyhedron promoter of the Autograph californica nuclear polyhedrosis virus (ACMNPV) followed by convenient restriction sites. For an easy selection of recombinant virus the beta-galactosidase gene from *E. coli* is inserted in the same orientation as the polyhedron promoter and is followed by the polyadenylation signal of the polyhedron gene. The polyhedron sequences are flanked at both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of pA2, such as pAc373, pVL941 and pAcIM1 provided, as those of skill readily will appreciate, that construction provides appropriately located signals for transcription, translation, trafficking and the like, such as an in-frame AUG and a signal peptide, as required. Such vectors are described in Luckow *et al., Virology 170*:31-39, among others.

The plasmid is digested with the restriction enzyme Bam HI and then is dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

Fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 cells are transformed with ligation mix and spread on culture plates. Bacteria are identified that contain the plasmid with the human DDCR gene by digesting DNA from individual colonies using BamHI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac DR4.

5 µg of the plasmid pBac DR4 is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al., Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBac DR4 are mixed in a sterile well of a microliter plate containing 50 µl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27 C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium

supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27 C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after serial dilution, the virus is added to the cells. After appropriate incubation, blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses is then resuspended in an Eppendorf tube containing 200 μl of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 C. A clone containing properly inserted DR4 is identified by DNA analysis including restriction mapping and sequencing. This is designated herein as V- DR4.

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V- DR4 at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Gaithersburg). 42 hours later, 5 gCi of ^{35}S-methionine and 5 μCi ^{35}S cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation, lysed and the labeled proteins are visualized by SDS-PAGE and autoradiography.

Example 4: Tissue distribution of DR4 gene expression

Northern blot analysis is carried out to examine DR4 gene (ATCC No. 97853) expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the DR4 protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for DR4 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 C overnight, and films developed according to standard procedures Expression of DR4 was detected in tissues enriched in lymphocytes including amniotic cells, heart, liver cancer, kidney, peripheral blood leukocytes, activated T-cell, K562 plus PMA, W138 cells, Th2 cells, human tonsils, and CD34 depleted buffy coat (cord blood). It can be envisaged that DR4 plays a role in lymphocyte homeostasis.

Example 5: DR4 Induced Apoptosis

Overexpression of Fas/APO-1 and TNFR-1 in mammalian cells mimics receptor activation (M. Muzio, *et al., Cell 85*, 817-827 (1996); M. P. Boldin, *et al., Cell 85*, 803-815 (1996)). Thus, this system was utilized to study the functional role of DR4. Transient expression of DR4 in MCF7 human breast carcinoma cells and 293 human embryonic kidney cells induced rapid apoptosis.

Cell death assays are performed essentially as previously described (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995); A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)). Briefly, MCF-7 human breast carcinoma clonal cell lines stably transfected with either vector alone or a CrmA expression construct (M. Tewari, *et al., J Biol Chem 270*, 3255-60 (1995)), are transiently transfected with pCMV-DR4-galatosidase (or pCMV-DR4-galactosidase (lacking the death domain)) in the presence of a ten-fold excess of pcDNA3 expression constructs encoding the indicated proteins using lipofectamine (GIBCO-BRL). 293 cells are likewise transfected using the $CaPO_4$ method. The ICE family inhibitor z-VAD-fmk (Enzyme Systems Products, Dublin, CA) is added to the cells at a concentration of 10μM, 5 hrs after transfection. 32 hours following transfection, cells are fixed and stained with X-Gal as previously described (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)).

The cells displayed morphological alterations typical of cells undergoing apoptosis, becoming rounded, condensed and detaching from the dish. Similar to TNFR-1 and Fas/APO-1 (M. Muzio, *et al., Cell 85*, 817-827 (1996); M. P. Boldin, *et al., Cell 85*, 803-815 (1996); M. Tewari, *et al., J Biol Chem 270*, 3255-60 (1995)), DR4-induced apoptosis was blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosures of all patents, patent applications, and publications referred to herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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GENTZ, REINER L.
DIXIT, VISHVA M.

(ii) TITLE OF INVENTION: Death Domain Containing Receptor-4

(iii) NUMBER OF SEQUENCES: 11

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 28-JAN-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 36,373
(C) REFERENCE/DOCKET NUMBER: PF355

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: (301) 309-8512

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 19..1422

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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                     1               5                   10

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 60                  65                  70                  75

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    125                 130                 135

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            335   340   345

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400 405 410

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CCCCACGAAG GACAGTGTAG ACTGCAGATT GCACCACTGC ACTCCCAGCC TGGGAACACA 1802

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GGCTCACATG CCAAAGGAAA ATCTGGTTCT CCCCTGAGCT GGCCTCCGTG TGTTTCCTTA 1922

TCATGGTGGT CAATTGGAGG TGTTAATTTG AATGGATTAA GGAACACCTA GAACACTGGT 1982

AAGGCATTAT TTCTGGGACA TTATTTCTGG GCATGTCTTC GAGGGTGTTT CCAGAGGGGA 2042

TTGGCATGCG ATCGGGTGGA CTGAGTGGAA AAGACCTACC CTTAATTTGG GGGGGCACCG 2102

TCCGACAGAC TGGGGAGCAA GATAGAAGAA AACAAAAAAA AAAAAAAAAA 2152

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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35 40 45

Gly Gly Gly Arg Gly Ala Leu Pro Thr Ser Met Gly Gln His Gly Pro
50 55 60

Ser Ala Arg Ala Arg Ala Gly Arg Ala Pro Gly Pro Arg Pro Ala Arg
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Glu Ala Ser Pro Arg Leu Arg Val His Lys Thr Phe Lys Phe Val Val
85 90 95

Val Gly Val Leu Leu Gln Val Val Pro Ser Ser Ala Ala Thr Ile Lys
100 105 110

Leu His Asp Gln Ser Ile Gly Thr Gln Gln Trp Glu His Ser Pro Leu
115 120 125

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130 135 140

Cys Asn Arg Cys Thr Glu Gly Val Gly Tyr Thr Asn Ala Ser Asn Asn
145 150 155 160

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165 170 175

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Gly Thr Phe Arg Asn Asp Asn Ser Ala Glu Met Cys Arg Lys Cys Ser
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Lys Cys Met Asp Arg Val Cys Phe Trp Arg Leu Gly Leu Leu Arg Gly
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Pro Gly Ala Glu Asp Asn Ala His Asn Glu Ile Leu Ser Asn Ala Asp
290 295 300

Ser Leu Ser Thr Phe Val Ser Glu Gln Gln Met Glu Ser Gln Glu Pro
305 310 315 320

Ala Asp Leu Thr Gly Val Thr Val Gln Ser Pro Gly Glu Ala Gln Cys
325 330 335

Leu Leu Gly Pro Ala Glu Ala Glu Gly Ser Gln Arg Arg Arg Leu Leu
340 345 350

Val Pro Ala Asn Gly Ala Asp Pro Thr Glu Thr Leu Met Leu Phe Phe
355 360 365

Asp Lys Phe Ala Asn Ile Val Pro Phe Asp Ser Trp Asp Gln Leu Met
370 375 380

Arg Gln Leu Asp Leu Thr Lys Asn Glu Ile Asp Val Val Arg Ala Gly
385 390 395 400

Thr Ala Gly Pro Gly Asp Ala Leu Tyr Ala Met Leu Met Lys Trp Val
405 410 415

Asn Lys Thr Gly Arg Asn Ala Ser Ile His Thr Leu Leu Asp Ala Leu
420 425 430

Glu Arg Met Glu Glu Arg His Ala Lys Glu Lys Ile Gln Asp Leu Leu
435 440 445

Val Asp Ser Gly Lys Phe Ile Tyr Leu Glu Asp Gly Thr Gly Ser Ala
450 455 460

Val Ser Leu Glu
465

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 669 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala
1 5 10 15

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
20 25 30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn
        35   40   45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
    50   55   60

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65   70   75   80

Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
                85   90   95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
            100   105   110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
        115   120   125

Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
    130   135   140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145   150   155   160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
                165   170   175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
            180   185   190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
        195   200   205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu
    210   215   220

Ser Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met
225   230   235   240

Thr Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu
                245   250   255

Ala Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu
            260   265   270

Gln Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys
        275   280   285

Glu Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys
    290   295   300

Thr Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser
305   310   315   320

Asp Ser Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val Met

325 330 335

Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala Arg
340 345 350

Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser Lys
355 360 365

Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn Leu
370 375 380

Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro Pro
385 390 395 400

Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro Asp
405 410 415

Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His Phe
420 425 430

Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly Leu
435 440 445

Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg Cys
450 455 460

Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp Pro
465 470 475 480

Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr Ser
485 490 495

Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp Leu
500 505 510

Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Val Lys Arg Lys Glu
515 520 525

Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly Ser His
530 535 540

Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu Ser Asp
545 550 555 560

Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met Thr Leu
565 570 575

Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu Ala Lys
580 585 590

Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu Gln Lys
595 600 605

Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys Glu Ala
610 615 620

Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys Thr Leu
625 630 635 640

Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser Asp Ser
645 650 655

Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
660 665

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 909 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Pro Leu Val Leu Leu Glu
1 5 10 15

Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro His
20 25 30

Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys Tyr
35 40 45

Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly
50 55 60

Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Asp Thr Asp Cys Arg
65 70 75 80

Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His
85 90 95

Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile
100 105 110

Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn
115 120 125

Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys
130 135 140

Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln
145 150 155 160

Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu
165 170 175

Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu
180 185 190

Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr
195 200 205

Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu Leu Ser
210 215 220

Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys Ser Lys
225 230 235 240

Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu Gly Glu
245 250 255

Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser Phe Ser
260 265 270

Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val Pro Ser
275 280 285

Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys Pro Asn
290 295 300

Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly Ala Asp
305 310 315 320

Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn Pro Leu
325 330 335

Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp Thr Asp
340 345 350

Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro Leu Arg
355 360 365

Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu Ile Asp
370 375 380

Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln Tyr Ser
385 390 395 400

Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala Thr Leu
405 410 415

Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly Cys Leu
420 425 430

Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro Pro Ala
435 440 445

Pro Ser Leu Leu Arg Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu

450 455 460

Pro Leu Val Leu Leu Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val
465 470 475 480

Ile Gly Leu Val Pro His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val
485 490 495

Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys
500 505 510

Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro
515 520 525

Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala
530 535 540

Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Glu
545 550 555 560

Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp
565 570 575

Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu
580 585 590

Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val
595 600 605

His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala
610 615 620

Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys
625 630 635 640

Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val
645 650 655

Lys Gly Thr Glu Asp Ser Gly Thr Thr Val Leu Leu Pro Leu Val Ile
660 665 670

Phe Phe Gly Leu Cys Leu Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr
675 680 685

Arg Tyr Gln Arg Trp Lys Ser Asp Leu Tyr Ser Ile Val Cys Gly Lys
690 695 700

Ser Thr Pro Glu Lys Glu Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro
705 710 715 720

Leu Ala Pro Asn Pro Ser Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr
725 730 735

Leu Gly Phe Ser Pro Val Pro Ser Ser Thr Phe Thr Ser Ser Ser Thr
740 745 750

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Tyr Thr Pro Gly Asp Cys Pro Asn Phe Ala Ala Pro Arg Arg Glu Val
        755                 760                 765

Ala Pro Pro Tyr Gln Gly Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala
    770                 775                 780

Ser Asp Pro Ile Pro Asn Pro Leu Gln Lys Trp Glu Asp Ser Ala His
785                 790                 795                 800

Lys Pro Gln Ser Leu Asp Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val
                805                 810                 815

Val Glu Asn Val Pro Pro Leu Arg Trp Lys Glu Phe Val Arg Arg Leu
            820                 825                 830

Gly Leu Ser Pro His Glu Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg
        835                 840                 845

Cys Leu Arg Glu Ala Gln Tyr Ser Met Leu Ala Thr Trp Arg Arg Arg
    850                 855                 860

Thr Pro Arg Arg Glu Ala Thr Leu Glu Leu Leu Gly Arg Val Leu Arg
865                 870                 875                 880

Asp Met Asp Leu Leu Gly Cys Leu Glu Asp Ile Glu Glu Ala Leu Cys
                885                 890                 895

Gly Pro Ala Ala Leu Pro Pro Ala Pro Ser Leu Leu Arg
            900                 905
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 833 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Glu Gln Arg Pro Arg Gly Cys Ala Ala Val Ala Ala Ala Leu Leu
1               5                   10                  15

Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
            20                  25                  30

Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
        35                  40                  45
```

Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
50 55 60

Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
65 70 75 80

Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
85 90 95

Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
100 105 110

Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
115 120 125

Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
130 135 140

Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
145 150 155 160

Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
165 170 175

Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
180 185 190

Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
195 200 205

Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
210 215 220

Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
225 230 235 240

Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp
245 250 255

Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
260 265 270

Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
275 280 285

Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
290 295 300

Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
305 310 315 320

Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
325 330 335

Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg

340 345 350

Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
355 360 365

Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
370 375 380

Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
385 390 395 400

Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
405 410 415

Pro Met Glu Gln Arg Pro Arg Gly Cys Ala Ala Val Ala Ala Ala Leu
420 425 430

Leu Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro
435 440 445

Arg Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys
450 455 460

Cys Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu
465 470 475 480

Pro Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu
485 490 495

Ala Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys
500 505 510

Asp Glu Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
515 520 525

Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
530 535 540

Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
545 550 555 560

Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
565 570 575

Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
580 585 590

Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
595 600 605

Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
610 615 620

Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
625 630 635 640

```
Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
                645                 650                 655

Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp
            660                 665                 670

Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
        675                 680                 685

Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
    690                 695                 700

Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
705                 710                 715                 720

Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
                725                 730                 735

Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
            740                 745                 750

Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
        755                 760                 765

Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
    770                 775                 780

Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
785                 790                 795                 800

Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
                805                 810                 815

Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
            820                 825                 830

Pro
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 426 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGCANAGGTN CGTACCTAGC TCACCTGCAA CCATCAAACT TNATGATCAA TCAATTGGCA 60

CACAGCAATG GGAAACATAG CCCTTTGGAA GANTTGTNTC CACCAGGATC TCATAGATCA 120

AAACATCCTG GGAGCCTGTT AACCGGTGCC CCAAAGGNTG GTCAAGGTCA AGGAATTGTT 180

NCGCCCTGGA AGTGAACATC GAGTGTNTCC ACAAAGGATT CAGGCAATGG GACATAAATA 240

TATGGGTGAA TTTTGGTTGT GAACTTTGGT TGNTCCCGTT GNTGTTGNTG GCTGTGCTGA 300

TTGTTTGTTG TTGCATCGGC TTCAGGTTNT GGAGGGGGAC CCAAGTGCAT GGACAGGGTG 360

TGTTTCTGGG GTTTGGGTCT CTTAGAGGGC NTGGGTTANG GCANGTTCAC AAGGGTTTTA 420

GCAANG 426

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGGGCTGAG GACAATGCTG ACNACGAGAT TCTGAGCAAC GCAGNACTNG CTGTCCACTT 60

TCGTCTNTGN GCAGCAAATG GAAAGCCAGG AGCCGGCAGA TTTGACAGGT GTCACTGTAC 120

AGTCCCCAGG GGAGGCACAG TGTCTGCTGG TGAGTTGGGG ACAGGCCCTT GCAAGACCTT 180

GTGAGGCAGG GGGTGAAGGC CATGNCTCGG CTTCNNNTGG TCAAAGGGGA AGTGGAGCCT 240

GAGGGAGATG GGACTTNAGG GGGACGGNGC TGCGTGGGGA AAAAGCAGCC ACCNTTTGAC 300

AAGGGGGACA GGCATTTTTN CAAATGTGTG CTTNTTGGT 339

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGGCATGCA TGATCAATCA ATTGGCAC 28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCGGGATCCG CCATCATGGC GCCACCACCA GCTAGA 36

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCGGGATCCT CACTCCAAGG ACACGGCAGA GCC 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCGGGATCCT CAATTATGTC CATTGCCTG 29

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence;

(b) nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence but lacking the amino terminal methionine;

(c) a nucleotide sequence encoding the mature DR4 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 24 to about 468 in Figure 1 (SEQ ID NO:2);

(d) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(e) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader but lacking the amino terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(f) a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(g) a nucleotide sequence that encodes the DR4 extracellular domain having the amino acid sequence at positions about 24 to about 238 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(h) a nucleotide sequence that encodes the DR4 transmembrane domain having the amino acid sequence at positions about 239 to about 264 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(i) a nucleotide sequence that encodes the DR4 intracellular domain having the amino acid sequence at positions about 265 to about 468 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(j) a nucleotide sequence that encodes the DR4 death domain domain having the amino acid sequence at positions about 379 to about 422 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853; and

(k) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above.

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figure 1 (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the DR4 polypeptide having the amino acid sequence in positions 2 - 468 of SEQ ID NO:2.

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the extracellular domain of the DR4 polypeptide having the amino acid sequence from about 24 to about 238 in SEQ ID NO:2.

5. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues n-468 of SEQ ID NO:2. where n is an integer in the range of 1-109:

(b) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues 1-m of S EQ ID NO:2, where m is an integer in the range of 221 - 468;

(c) a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues n-m of SEQ ID NO:2, where n and m are integers as defined respectively in (a) and (b) above; and

(d) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete DR4 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853 wherein said portion excludes from 1 to about 108 amino acids from the amino terminus of said complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(e) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete DR4 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853 wherein said portion excludes from 1 to about 249 amino acids from the carboxy terminus of said complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853; and

(f) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete DR4 amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853 wherein said portion include a combination of any of the amino terminal and carboxy terminal deletions in (d) and (e), above.

6. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 97853.

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the DR4 polypeptide having the complete amino acid sequence excepting the N-terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853.

8. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the extracellular domain of the DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853.

9. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

10. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a DR4 polypeptide having an amino acid sequence in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k) of claim 1.

11. The isolated nucleic acid molecule of claim 10, which encodes an epitope-bearing portion of a DR4 polypeptide wherein the amino acid sequence of said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about 35 to about 92 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 114 to about 160 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 169 to about 240 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 267 to about 298 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 330 to about 364 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 391 to about 404 of SEQ ID NO:2, and a polypeptide comprising amino acid residues from about 418 to about 465 of SEQ ID NO:2.

12. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

13. A recombinant vector produced by the method of claim 12.

14. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 13 into a host cell.

15. A recombinant host cell produced by the method of claim 14.

16. A recombinant method for producing a DR4 polypeptide, comprising culturing the recombinant host cell of claim 15 under conditions such that said polypeptide is expressed and recovering said polypeptide.

17. An isolated DR4 polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence;

(b) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence but lacking the amino terminal methionine;

(c) the amino acid sequence of the mature DR4 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 24 to about 468 in Figure 1 (SEQ ID NO:2);

(d) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(e) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence including the leader but lacking the amino terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(f) the amino acid sequence of the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(g) the amino acid sequence of the DR4 extracellular domain having the amino acid sequence at positions about 24 to about 238 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(h) the amino acid sequence of the DR4 transmembrane domain having the amino acid sequence at positions about 239 to about 264 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(i) the amino acid sequence of the DR4 intracellular domain having the amino acid sequence at positions about 265 to about 468 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853; and

(j) the amino acid sequence of the DR4 death domain having the amino acid sequence at positions about 379 to about 422 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853.

18. An isolated polypeptide comprising an epitope-bearing portion of the DR4 protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about 35 to about 92 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 114 to about 160 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 169 to about 240 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 267 to about 298 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 330 to about 364 of SEQ ID NO:2, a polypeptide comprising amino acid residues from about 391 to about 404 of SEQ ID NO:2, and a polypeptide comprising amino acid residues from about 418 to about 465 of SEQ ID NO:2.

19. An isolated antibody that binds specifically to a DR4 polypeptide of claim 17.

20. An isolated nucleic acid molecule comprising a polynucleotide encoding a DR4 polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence;

(b) nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence but lacking the amino terminal methionine;

(c) a nucleotide sequence encoding the mature DR4 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 24 to about 468 in Figure 1 (SEQ ID NO:2);

(d) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(e) a nucleotide sequence encoding the full-length DR4 polypeptide having the complete amino acid sequence including the leader but lacking the amino terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(f) a nucleotide sequence encoding the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(g) a nucleotide sequence that encodes the DR4 extracellular domain having the amino acid sequence at positions about 24 to about 238 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(h) a nucleotide sequence that encodes the DR4 transmembrane domain having the amino acid sequence at positions about 239 to about 264 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(i) a nucleotide sequence that encodes the DR4 intracellular domain having the amino acid sequence at positions about 265 to about 468 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(j) a nucleotide sequence that encodes the DR4 death domain domain having the amino acid sequence at positions about 379 to about 422 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853; and

(k) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above.

21. An isolated DR4 polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has a sequence selected from the group consisting of:

(a) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence;

(b) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence but lacking the amino terminal methionine;

(c) the amino acid sequence of the mature DR4 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 24 to about 468 in Figure 1 (SEQ ID NO:2);

(d) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(e) the amino acid sequence of the full-length DR4 polypeptide having the complete amino acid sequence including the leader but lacking the amino terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(f) the amino acid sequence of the mature DR4 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97853;

(g) the amino acid sequence of the DR4 extracellular domain having the amino acid sequence at positions about 24 to about 238 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(h) the amino acid sequence of the DR4 transmembrane domain having the amino acid sequence at positions about 239 to about 264 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853;

(i) the amino acid sequence of the DR4 intracellular domain having the amino acid sequence at positions about 265 to about 468 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853; and

(j) the amino acid sequence of the DR4 death domain having the amino acid sequence at positions about 379 to about 422 of SEQ ID NO:2, or as encoded by ATCC Deposit No. 97853.

Abstract

The present invention relates to novel Death Domain Containing Receptor-4 (DR4) proteins which are members of the tumor necrosis factor (TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding the human DR4 proteins. DR4 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR4 activity.

FIG. 1A

```
           10                             30                             50
TTCGGGCACGAGGGCAGGATGGCGCCACCACCAGCTAGAGTACATCTAGGTGCGTTCCTG
                      M  A  P  P  P  A  R  V  H  L  G  A  F  L
           70                             90                            110
GCAGTGACTCCGAATCCCGGGAGCGCAGCGAGTGGGACAGAGGCAGCCGCGGCCACACCC
A  V  T  P  N  P  G  S  A  A  S  G  T  E  A  A  A  A  T  P
          130                            150                            170
AGCAAAGTGTGGGGCTCTTCCGCGGGGAGGATTGAACCACGAGGCGGGGGCCGAGGAGCG
S  K  V  W  G  S  S  A  G  R  I  E  P  R  G  G  G  R  G  A
          190                            210                            230
CTCCCTACCTCCATGGGACAGCACGGACCCAGTGCCCGGGCCCGGGCAGGGCGCGCCCCA
L  P  T  S  M  G  Q  H  G  P  S  A  R  A  R  A  G  R  A  P
          250                            270                            290
GGACCCAGGCCGGCGCGGGAAGCCAGCCCTCGGCTCCGGGTCCACAAGACCTTCAAGTTT
G  P  R  P  A  R  E  A  S  P  R  L  R  V  H  K  T  F  K  F
          310                            330                            350
GTCGTCGTCGGGGTCCTGCTGCAGGTCGTACCTAGCTCAGCTGCAACCATCAAACTTCAT
V  V  V  G  V  L  L  Q  V  V  P  S  S  A  A  T  I  K  L  H
          370                            390                            410
GATCAATCAATTGGCACACAGCAATGGGAACATAGCCCTTTGGGAGAGTTGTGTCCACCA
D  Q  S  I  G  T  Q  Q  W  E  H  S  P  L  G  E  L  C  P  P
          430                            450                            470
GGATCTCATAGATCAGAACGTCCTGGAGCCTGTAACCGGTGCACAGAGGGTGTGGGTTAC
G  S  H  R  S  E  R  P  G  A  C  N  R  C  T  E  G  V  G  Y
          490                            510                            530
ACCAATGCTTCCAACAATTTGTTTGCTTGCCTCCCATGTACAGCTTGTAAATCAGATGAA
T  N  A  S  N  N  L  F  A  C  L  P  C  T  A  C  K  S  D  E
          550                            570                            590
GAAGAGAGAAGTCCCTGCACCACGACCAGGAACACAGCATGTCAGTGCAAACCAGGAACT
E  E  R  S  P  C  T  T  T  R  N  T  A  C  Q  C  K  P  G  T
          610                            630                            650
TTCCGGAATGACAATTCTGCTGAGATGTGCCGGAAGTGCAGCACAGGGTGCCCCAGAGGG
F  R  N  D  N  S  A  E  M  C  R  K  C  S  T  G  C  P  R  G
          670                            690                            710
ATGGTCAAGGTCAAGGATTGTACGCCCTGGAGTGACATCGAGTGTGTCCACAAAGAATCA
M  V  K  V  K  D  C  T  P  W  S  D  I  E  C  V  H  K  E  S
          730                            750                            770
GGCAATGGACATAATATATGGGTGATTTTGGTTGTGACTTTGGTTGTTCCGTTGCTGTTG
G  N  G  H  N  I  W  V  I  L  V  V  T  L  V  V  P  L  L  L
                ************************************************

          790                            810                            830
GTGGCTGTGCTGATTGTCTGTTGTTGCATCGGCTCAGGTTGTGGAGGGGACCCCAAGTGC
V  A  V  L  I  V  C  C  C  I  G  S  G  C  G  G  D  P  K  C
****************************************

          850                            870                            890
ATGGACAGGGTGTGTTTCTGGCGCTTGGGTCTCCTACGAGGGCCTGGGGCTGAGGACAAT
M  D  R  V  C  F  W  R  L  G  L  L  R  G  P  G  A  E  D  N
          910                            930                            950
GCTCACAACGAGATTCTGAGCAACGCAGACTCGCTGTCCACTTTCGTCTCTGAGCAGCAA
A  H  N  E  I  L  S  N  A  D  S  L  S  T  F  V  S  E  Q  Q
          970                            990                           1010
ATGGAAAGCCAGGAGCCGGCAGATTTGACAGGTGTCACTGTACAGTCCCCAGGGGAGGCA
M  E  S  Q  E  P  A  D  L  T  G  V  T  V  Q  S  P  G  E  A
```

FIG. 1B

1030 1050 1070
CAGTGTCTGCTGGGACCGGCAGAAGCTGAAGGGTCTCAGAGGAGGAGGCTGCTGGTTCCA
Q C L L G P A E A E G S Q R R R L L V P
1090 1110 1130
GCAAATGGTGCTGACCCCACTGAGACTCTGATGCTGTTCTTTGACAAGTTTGCAAACATC
A N G A D P T E T L M L F F D K F A N I
1150 1170 1190
GTGCCCTTTGACTCCTGGGACCAGCTCATGAGGCAGCTGGACCTCACGAAAAATGAGATC
V P F D *S W D Q L M R Q L D L T K N E I*
1210 1230 1250
GATGTGGTCAGAGCTGGTACAGCAGGCCCAGGGGATGCCTTGTATGCAATGCTGATGAAA
D V V R A G T A G P G D A L Y A M L M K
1270 1290 1310
TGGGTCAACAAAACTGGACGGAACGCCTCGATCCACACCCTGCTGGATGCCTTGGAGAGG
W V N K T G R N A S I H T L L D A L E R
1330 1350 1370
ATGGAAGAGAGACATGCAAAAGAGAAGATTCAGGACCTCTTGGTGGACTCTGGAAAGTTC
M E E R H A K E K I Q D L L V D S G K F
1390 1410 1430
ATCTACTTAGAAGATGGCACAGGCTCTGCCGTGTCCTTGGAGTGAAAGACTCTTTTTACC
I Y L E D G T F S A V S L E
1450 1470 1490
AGAGGTTTCCTCTTAGGTGTTAGGAGTTAATACATATTAGGTTTTTTTTTTTTTTAACAT
1510 1530 1550
GTATACAAAGTAAATTCTTAGCCACGTGTATTGGCTCCTGCCTGTAATCCCATCACTTTG
1570 1590 1610
GGAGGCTGACGCCGGTGGATCCACTTGAGGTCCGAAGTTCCAAGACCAGCCCTGAACCAA
1630 1650 1670
CATCGTGGAAATGCCCGTCTTTTACAAAAAAATACCAAAAATTCAACTGGAATGTGCATG
1690 1710 1730
GTGTGTGCCATCATTTCCTCGGCTAACTACGGGAGGTCTGAGGCCAGGAGAATCCACTTG
1750 1770 1790
AACCCCACGAAGGACAGTGTAGACTGCAGATTGCACCACTGCACTCCCAGCCTGGGAACA
1810 1830 1850
CAGAGCAAGACTCTGTCTCAAGATAAAATAAAATAAACTTGAAAGAATTATTGCCCGACT
1870 1890 1910
GAGGCTCACATGCCAAAGGAAAATCTGGTTCTCCCCTGAGCTGGCCTCCGTGTGTTTCCT
1930 1950 1970
TATCATGGTGGTCAATTGGAGGTGTTAATTTGAATGGATTAAGGAACACCTAGAACACTG
1990 2010 2030
GTAAGGCATTATTTCTGGACATTATTTCTGGGCATGTCTTCGAGGGTGTTTCCAGAGGG
2050 2070 2090
GATTGGCATGCGATCGGGTGGACTGAGTGGAAAAGACCTACCCTTAATTTGGGGGGCAC
2110 2130 2150
CGTCCGACAGACTGGGGAGCAAGATAGAAGAAAACAAAAAAAAAAAAAAAA

FIG. 2A

```
1   M---------LG------------------------IMT-  h Fas protein
1   MGLSTVPDLLLPLVLLELLVGIYPSGVIGLVPH-------  h TNFR I Protein
1   MEQRPRGCAAVAAALLLVLLGARAQG--------------  DR3 protein
1   MAPPPARVHLGAFLAVTPNPGSAASGTEAAAATPSKVWGS  DR4 protein

7   -------------LLPLVLT---SV-----ARL-SSKSVN  h Fas protein
34  -LGDREKRDSVCPQGKYIHPQNNSICCTKCHKGTYLYNDC  h TNFR I Protein
27  --GTRSPR-CDCA-GDF-HKKIGLFCCRGCPAGHYLKAPC  DR3 protein
41  SAGRIEPRGGGRGALPTSMGQHGPS-----ARARAGRAPG  DR4 protein

25  AQVTDINSKGLELRKTVTTVETQNLEG----------LHH  h Fas protein
73  PGPGQDTDCRECESGSFTASENHLR-HCLSCSKCRKEMGQ  h TNFR I Protein
62  TEPCGNSTCLVCPQDTFLAWENHHNSECARCQACDEQASQ  DR3 protein
76  PRPAREASPRLRVHKTFKFVVVGVLLQVVPSSAATIKLHD  DR4 protein

55  DG----QFCHKP----CPPGERKARDCTVNGDEPDCVPCQ  h Fas protein
112 VEISS-----------CTVDRDTVCGC----RKNQYRHYW  h TNFR I Protein
102 VALEN-----------CSAVADTRCGC----KPGWFVEC-  DR3 protein
116 QSIGTQQWEHSPLGELCPPGSHRS------ERPGACNRCT  DR4 protein

87  EGKEYTDKAHFSSKCRRCRLCDEGHGLEVEINCTRTQNTK  h Fas protein
137 SENLFQC-----FNCSLCLN-GTVH-----LSCQEKQNTV  h TNFR I Protein
126 --QVSQCVSSSPFYCQPCLDCGALHR-HTRLLCSRRDTDC  DR3 protein
150 EGVGYTNASNNLFACLPCTACKSDE--EERSPCTTTRNTA  DR4 protein

127 CRCKPNFFCNSTVCEHCDPCTK-CEHGIIK--ECTLTSNT  h Fas protein
166 CTCHAGFFLRE---NECVSCSN-CKKSLECTKLCLPQIEN  h TNFR I Protein
163 GTCLPGFYEHG---DGCVSCPT-STLG-SCPERCAAVCGW  DR3 protein
188 CQCKPGTFRNDNSAEMCRKCSTGCPRGMVKVKDCTPWSDI  DR4 protein

164 KC-KEEGSRSNLGWLCL-------LLLPIPLIV-------  h Fas protein
202 VKGTEDSGTTVLLPLVIFFGLCLLSLLFIGLM--------  h TNFR I Protein
198 RQ--------MFWVQVLLAGLVVPLLLGATLT--------  DR3 protein
228 ECVHKESGNGHNIWVILVVTLVVPLLLVAVLIVCCCIGSG  DR4 protein

189 -------------W--------------------------  h Fas protein
234 ------YRYQR--WKSKLYSIVCGKSTPEKEGELEGTTTK  h TNFR I Protein
222 ------YTYRHC-WPHKPL-VTADEAGMEALTPPPATHLS  DR3 protein
268 CGGDPKCMDRVCFWRLGLLRGPGAEDNAHNEILSNADSLS  DR4 protein

190 --VKRKEVQKT----------------------------C  h Fas protein
266 PLAPNPSFSPTPGFTPTLGFSPVPSSTFTSSSTYTPGD-C  h TNFR I Protein
254 PLDSAHTLLAPPDSSEKICTVQLVGNSWTPGYPETQEALC  DR3 protein
308 TFVSEQQMESQEPADLTGVTVQSPG-----------EAQC  DR4 protein

200 ----------RKHRKENQGSHESPTLNPETVAINLS----  h Fas protein
305 PNFAAPRREVAPPYQGADPILATALASDPIPNPLQKWEDS  h TNFR I Protein
294 PQVTWSWDQL--PSRALGPAAAPTLSP----------ESP  DR3 protein
337 ----------LLGPAEAEGSQRRRLLVPANGADPTE----  DR4 protein

226 --------DVDLSKYITTIAGVMTLSQVKGFVRKNGVNEA  h Fas protein
345 AHKPQSLDTDDPATLYAVVENVPPL-RWKEFVRRLGLSDH  h TNFR I Protein
322 AGSPAMMLQPGPQ-LYDVMDAVPAR-RWKEFVRTLGLREA  DR3 protein
363 --------TLML--FFDKFANIVPFDSWDQLMRQLDLTKN  DR4 protein

258 KIDEIKNDNVQDTAEQKVQLLRNWHQLHGKKEA-YDTLIK  h Fas protein
384 EIDRLELQNGRCLREAQYSMLATWRRRTPRREATLELLGR  h TNFR I Protein
360 EIEAVEVEIGR-FRDQQYEMLKRWRQQQP---AGLGAVYA  DR3 protein
393 EIDVVRAGTA-GPGDALYAMLMKWVNKTGRNAS-IHTLLD  DR4 protein

297 DLKKANLCTLAEKIQTIILKDITSDSENSNFRNEIQSLV  h Fas protein
424 VLRDMDLLGCLEDIEEAL-------CGPAALPPAPSLLR  h TNFR I Protein
396 ALERMGLDGCVEDL-------------RSRLQRGP  DR3 protein
431 ALERMEERHAKEKIQDLLVDSGKFIYLEDGTGSAVSLE  DR4 protein
```

FIG. 2B

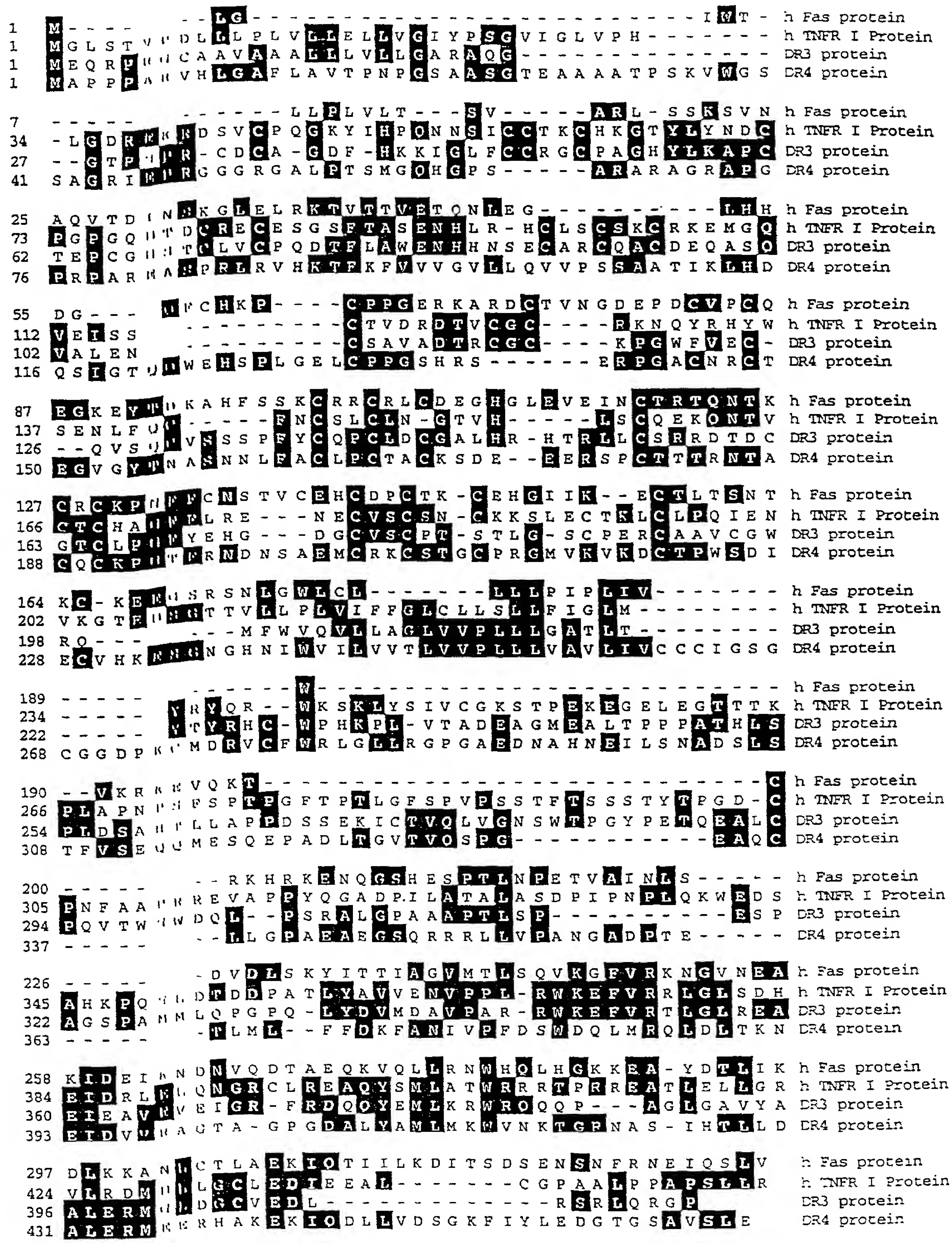
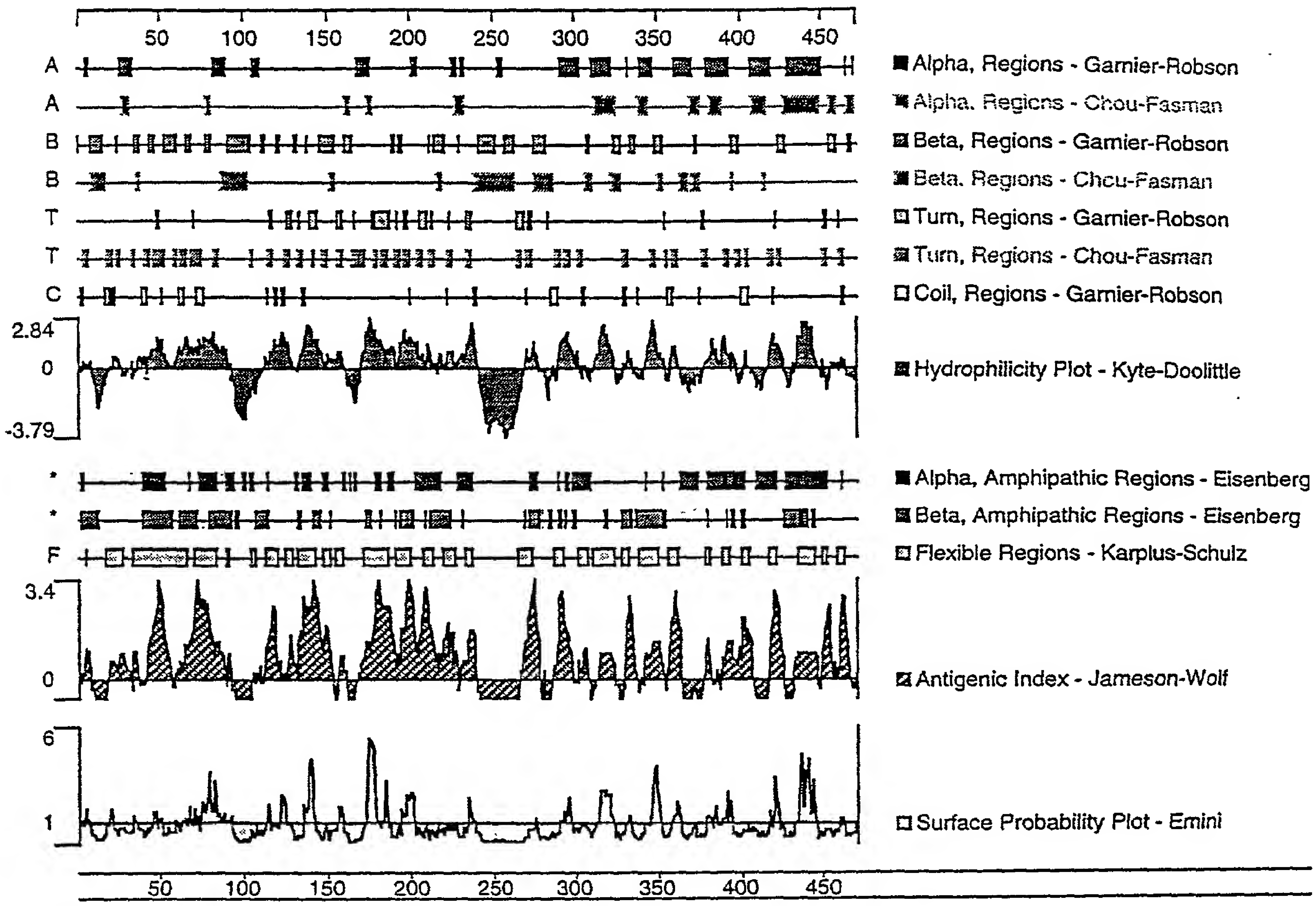


Figure 3



50
100
150
200
250
300
350
400
450
A
A
B
B
T
T
C
2.84
0
-3.79
F
3.4
0
6
1
Alpha, Regions - Garnier-Robson
Alpha, Regions - Chou-Fasman
Beta, Regions - Garnier-Robson
Beta, Regions - Chou-Fasman
Turn, Regions - Garnier-Robson
Turn, Regions - Chou-Fasman
Coil, Regions - Garnier-Robson
Hydrophilicity Plot - Kyte-Doolittle
Alpha, Amphipathic Regions - Eisenberg
Beta, Amphipathic Regions - Eisenberg
Flexible Regions - Karplus-Schulz
Antigenic Index - Jameson-Wolf
Surface Probability Plot - Emini

FIG. 4A

HTOIY07R

```
  1 GGCANAGGTN CGTACCTAGC TCACCTGCAA CCATCAAACT TNATGATCAA
 51 TCAATTGGCA CACAGCAATG GGAAACATAG CCCTTTGGAA GANTTGTNTC
101 CACCAGGATC TCATAGATCA AAACATCCTG GGAGCCTGTT AACCGGTGCC
151 CCAAAGGNTG GTCAAGGTCA AGGAATTGTT NCGCCCTGGA AGTGAACATC
201 GAGTGTNTCC ACAAAGGATT CAGGCAATGG GACATAAATA TATGGGTGAA
251 TTTTGGTTGT GAACTTTGGT TGNTCCCGTT GNTGTTGNTG GCTGTGCTGA
301 TTGTTTGTTG TTGCATCGGC TTCAGGTTNT GGAGGGGGAC CCAAGTGCAT
351 GGACAGGGTG TGTTTCTGGG GTTTGGGTCT CTTAGAGGGC NTGGGTTANG
401 GCANGTTCAC AAGGGTTTTA GCAANG
```

FIG. 4B

HTXEY80R

```
  1 TGGGGCTGAG GACAATGCTG ACNACGAGAT TCTGAGCAAC GCAGNACTNG
 51 CTGTCCACTT TCGTCTNTGN GCAGCAAATG GAAAGCCAGG AGCCGGCAGA
101 TTTGACAGGT GTCACTGTAC AGTCCCCAGG GGAGGCACAG TGTCTGCTGG
151 TGAGTTGGGG ACAGGCCCTT GCAAGACCTT GTGAGGCAGG GGGTGAAGGC
201 CATGNCTCGG CTTCNNNTGG TCAAAGGGGA AGTGGAGCCT GAGGGAGATG
251 GGACTTNAGG GGGACGGNGC TGCGTGGGGA AAAAGCAGCC ACCNTTTGAC
301 AAGGGGGACA GGCATTTTTN CAAATGTGTG CTTNTTGGT
```

Figure 5A

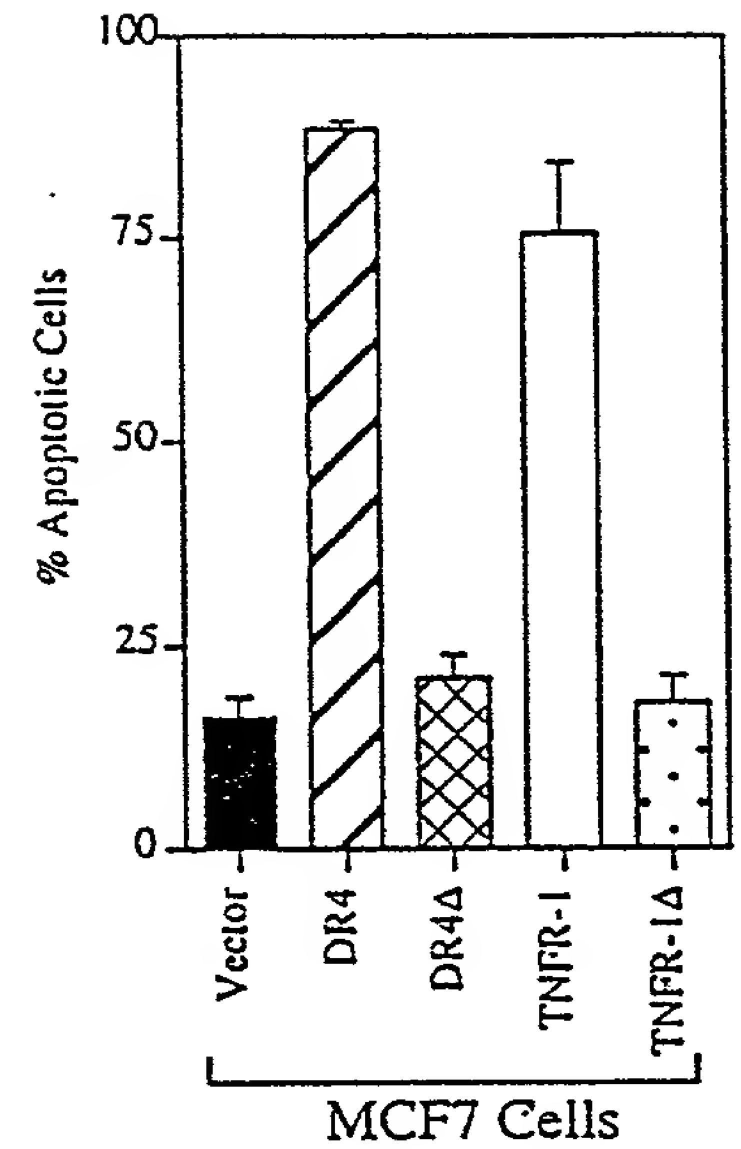


Figure 5B

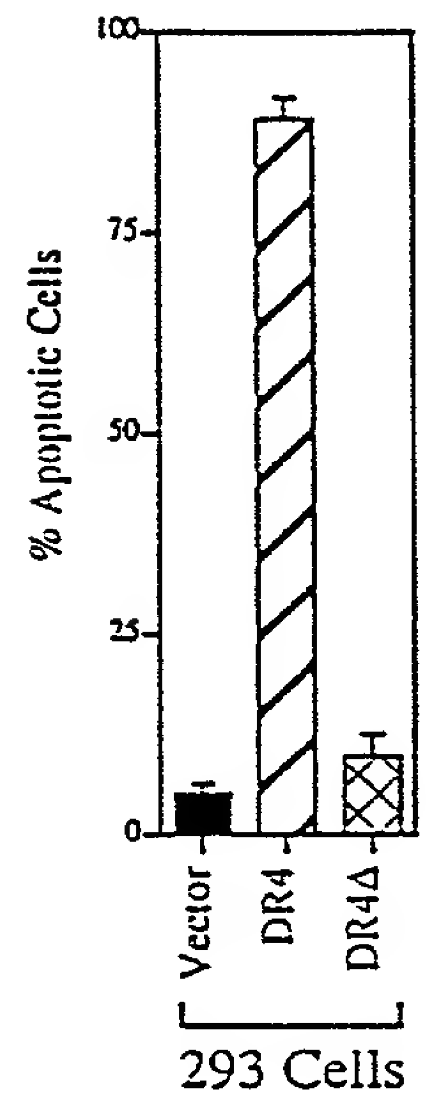


Figure 5C

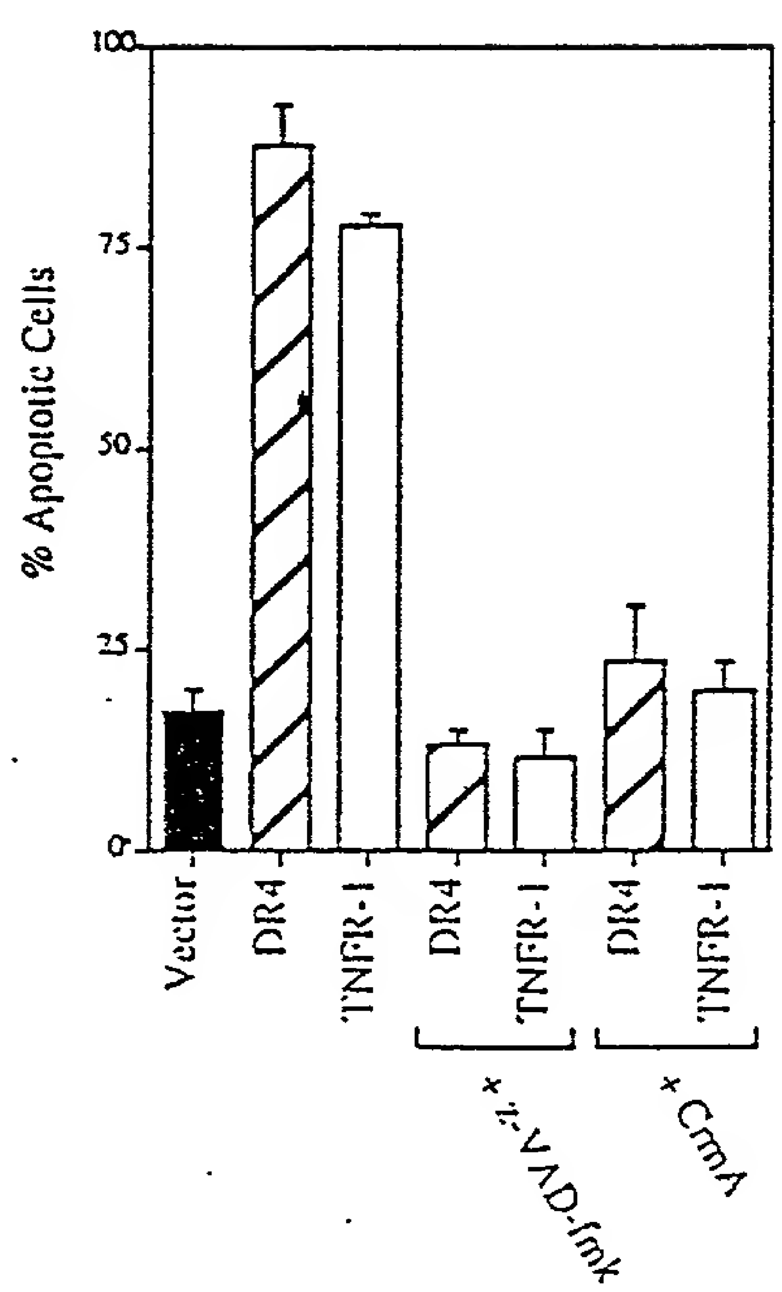


Figure 6A

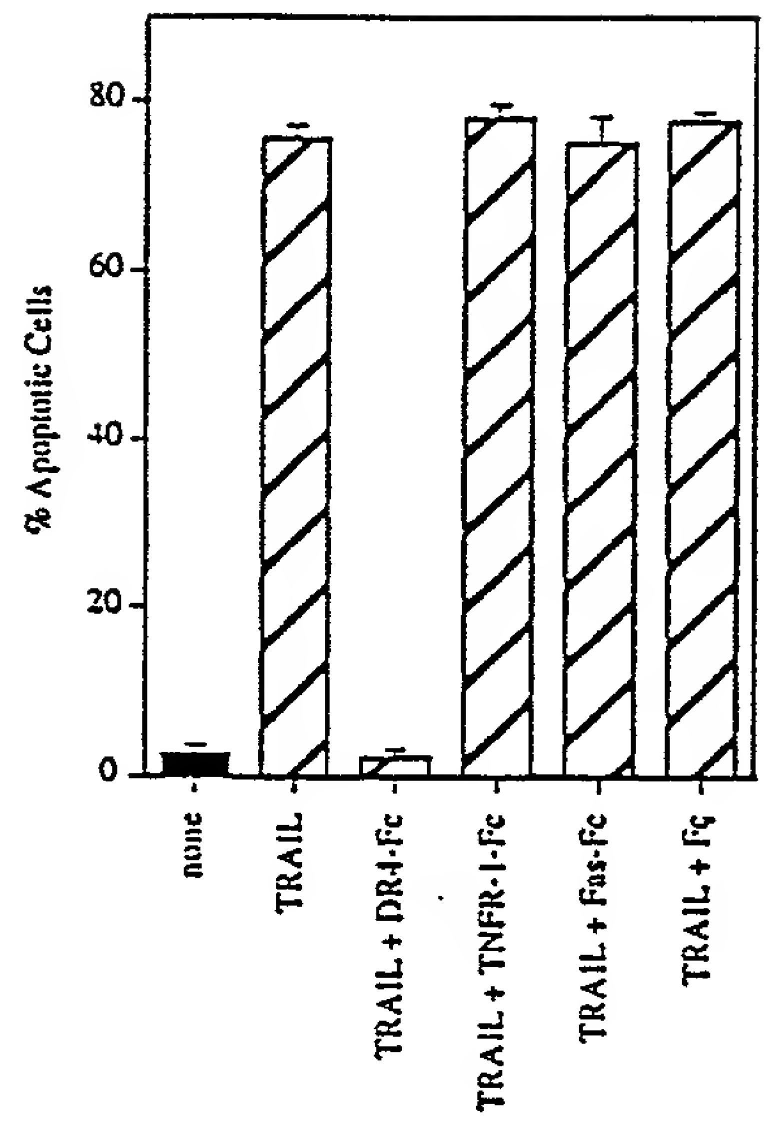
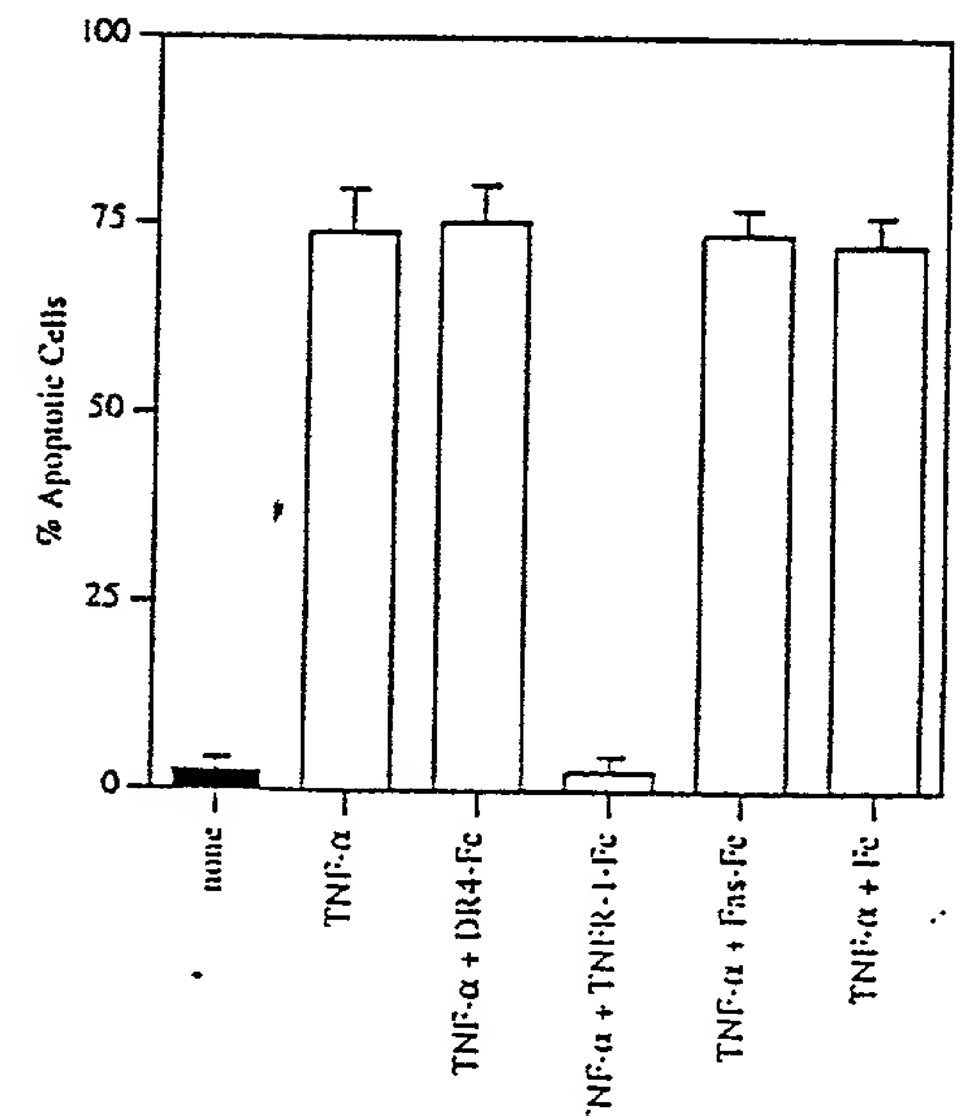


Figure 6B



DECLARATION FOR PATENT APPLICATION

As a below named inventor, I declare that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Death Domain Containing Receptor-4

the specification of which was filed on January 27, 1998 as Application Serial No. 09/013,895.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below , and have also identified below any foreign application for patent or inventor's certificate , or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

Priority Claimed
Yes No

(Number) (Country) (Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Serial No.)	(Filing Date)
60/035,722	01/28/97
60/037,829	02/05/97

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(b) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.) (Filing Date) (Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Robert H. Benson (Reg. No. 30,446), A. Anders Brookes (Reg. No. 36,373) and James H. Davis (Reg. No. 40,582) of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20878.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of additional joint inventor: Jian Ni

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Inventor's signature: ______ Date: 2/25/98
Residence: 22400 Rolling Hills Road, Laytonsville, MD 20882 Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: James G. Pan

Inventor's signature: ______ Date: ______
Residence: ______ Citizenship: ______
Post Office Address: same as above

Full name of first joint inventor: Reiner L. Gentz

Inventor's signature: ______ Date: 2-19-98
Residence: 13404 Fairland Park Drive, Silver Spring, MD 20904 Citizenship: DE
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Full name of additional joint inventor: Vishva M. Dixit

Inventor's signature: ______ Date: ______
Residence: ______ Citizenship: ______
Post Office Address: same as above

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I declare that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Death Domain Containing Receptor-4

the specification of which was filed on January 27, 1998 as Application Serial No. 09/013,895.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below , and have also identified below any foreign application for patent or inventor's certificate , or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

Priority Claimed
Yes No

(Number) (Country) (Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Serial No.)	(Filing Date)
60/035,722	01/28/97
60/037,829	02/05/97

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(b) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.) (Filing Date) (Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Robert H. Benson (Reg. No. 30,446), A. Anders Brookes (Reg. No. 36,373) and James H. Davis (Reg. No. 40,582) of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20878.

I hereby declare t a statements made herein of my o owledge are true and that all statements made on information and belief are believed to be t e; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of additional joint inventor: Jian Ni

Inventor's signature: ______________________ Date: __________
Residence: 5502 Manorfield Road, Rockville, MD 20853 Citizenship: CN
Post Office Address: same as above

Full name of additional joint inventor: Craig A. Rosen

Inventor's signature: ______________________ Date: __________
Residence: 22400 Rolling Hills Road, Laytonsville, MD 20882 Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: James G. Pan

Inventor's signature: G. Pan Date: Mar. 4/98
Residence: 4124 Green Meadows BLVD, Apt 11, Ypsilanti, Citizenship: Canadian
Post Office Address: same as above MI 48197 (CANADA)

Full name of first joint inventor: Reiner L. Gentz

Inventor's signature: ______________________ Date: __________
Residence: 13404 Fairland Park Drive, Silver Spring, MD 20904 Citizenship: DE
Post Office Address: same as above

Full name of additional joint inventor: Vishva M. Dixit

Inventor's signature: Vishva Dixit Date: Mar 13/98
Residence: 26750 Shady Oaks Court; Los Altos Hills Citizenship: USA
Post Office Address: same as above CA 94022

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ni, Jian
Rosen, Craig A.
Pan, James G.
Gentz, Reiner L.
Dixit, Vishva M.

(ii) TITLE OF INVENTION: Death Domain Containing Receptor 4 (DR4: Death Receptor 4), Member of the TNF-Receptor Superfamily and Binding to Trail (AP02-L)

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: HUMAN GENOME SCIENCES, INC.
(B) STREET: 9410 KEY WEST AVENUE
(C) CITY: ROCKVILLE
(D) STATE: MD
(E) COUNTRY: US
(F) ZIP: 20850

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: HEREWITH
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 09/013,895
(B) FILING DATE: 27-JAN-1998
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: STEFFE, ERIC K.
(B) REGISTRATION NUMBER: 36,688
(C) REFERENCE/DOCKET NUMBER: 1488.1300004

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202) 371-2600
(B) TELEFAX: (202)371-2540

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 19..1422

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
TTCGGGCACG AGGGCAGG ATG GCG CCA CCA CCA GCT AGA GTA CAT CTA GGT        51
                    Met Ala Pro Pro Pro Ala Arg Val His Leu Gly
                     1               5                  10

GCG TTC CTG GCA GTG ACT CCG AAT CCC GGG AGC GCA GCG AGT GGG ACA        99
Ala Phe Leu Ala Val Thr Pro Asn Pro Gly Ser Ala Ala Ser Gly Thr
            15                  20                  25

GAG GCA GCC GCG GCC ACA CCC AGC AAA GTG TGG GGC TCT TCC GCG GGG       147
Glu Ala Ala Ala Ala Thr Pro Ser Lys Val Trp Gly Ser Ser Ala Gly
        30                  35                  40

AGG ATT GAA CCA CGA GGC GGG GGC CGA GGA GCG CTC CCT ACC TCC ATG       195
Arg Ile Glu Pro Arg Gly Gly Gly Arg Gly Ala Leu Pro Thr Ser Met
    45                  50                  55

GGA CAG CAC GGA CCC AGT GCC CGG GCC CGG GCA GGG CGC GCC CCA GGA       243
Gly Gln His Gly Pro Ser Ala Arg Ala Arg Ala Gly Arg Ala Pro Gly
 60                  65                  70                  75

CCC AGG CCG GCG CGG GAA GCC AGC CCT CGG CTC CGG GTC CAC AAG ACC       291
Pro Arg Pro Ala Arg Glu Ala Ser Pro Arg Leu Arg Val His Lys Thr
                80                  85                  90

TTC AAG TTT GTC GTC GTC GGG GTC CTG CTG CAG GTC GTA CCT AGC TCA       339
Phe Lys Phe Val Val Val Gly Val Leu Leu Gln Val Val Pro Ser Ser
            95                 100                 105

GCT GCA ACC ATC AAA CTT CAT GAT CAA TCA ATT GGC ACA CAG CAA TGG       387
Ala Ala Thr Ile Lys Leu His Asp Gln Ser Ile Gly Thr Gln Gln Trp
        110                 115                 120

GAA CAT AGC CCT TTG GGA GAG TTG TGT CCA CCA GGA TCT CAT AGA TCA       435
Glu His Ser Pro Leu Gly Glu Leu Cys Pro Pro Gly Ser His Arg Ser
    125                 130                 135

GAA CGT CCT GGA GCC TGT AAC CGG TGC ACA GAG GGT GTG GGT TAC ACC       483
Glu Arg Pro Gly Ala Cys Asn Arg Cys Thr Glu Gly Val Gly Tyr Thr
140                 145                 150                 155

AAT GCT TCC AAC AAT TTG TTT GCT TGC CTC CCA TGT ACA GCT TGT AAA       531
Asn Ala Ser Asn Asn Leu Phe Ala Cys Leu Pro Cys Thr Ala Cys Lys
                160                 165                 170

TCA GAT GAA GAA GAG AGA AGT CCC TGC ACC ACG ACC AGG AAC ACA GCA       579
Ser Asp Glu Glu Glu Arg Ser Pro Cys Thr Thr Thr Arg Asn Thr Ala
            175                 180                 185

TGT CAG TGC AAA CCA GGA ACT TTC CGG AAT GAC AAT TCT GCT GAG ATG       627
Cys Gln Cys Lys Pro Gly Thr Phe Arg Asn Asp Asn Ser Ala Glu Met
        190                 195                 200

TGC CGG AAG TGC AGC ACA GGG TGC CCC AGA GGG ATG GTC AAG GTC AAG       675
Cys Arg Lys Cys Ser Thr Gly Cys Pro Arg Gly Met Val Lys Val Lys
```

205 210 215

GAT TGT ACG CCC TGG AGT GAC ATC GAG TGT GTC CAC AAA GAA TCA GGC 723
Asp Cys Thr Pro Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly
220 225 230 235

AAT GGA CAT AAT ATA TGG GTG ATT TTG GTT GTG ACT TTG GTT GTT CCG 771
Asn Gly His Asn Ile Trp Val Ile Leu Val Val Thr Leu Val Val Pro
240 245 250

TTG CTG TTG GTG GCT GTG CTG ATT GTC TGT TGT TGC ATC GGC TCA GGT 819
Leu Leu Leu Val Ala Val Leu Ile Val Cys Cys Cys Ile Gly Ser Gly
255 260 265

TGT GGA GGG GAC CCC AAG TGC ATG GAC AGG GTG TGT TTC TGG CGC TTG 867
Cys Gly Gly Asp Pro Lys Cys Met Asp Arg Val Cys Phe Trp Arg Leu
270 275 280

GGT CTC CTA CGA GGG CCT GGG GCT GAG GAC AAT GCT CAC AAC GAG ATT 915
Gly Leu Leu Arg Gly Pro Gly Ala Glu Asp Asn Ala His Asn Glu Ile
285 290 295

CTG AGC AAC GCA GAC TCG CTG TCC ACT TTC GTC TCT GAG CAG CAA ATG 963
Leu Ser Asn Ala Asp Ser Leu Ser Thr Phe Val Ser Glu Gln Gln Met
300 305 310 315

GAA AGC CAG GAG CCG GCA GAT TTG ACA GGT GTC ACT GTA CAG TCC CCA 1011
Glu Ser Gln Glu Pro Ala Asp Leu Thr Gly Val Thr Val Gln Ser Pro
320 325 330

GGG GAG GCA CAG TGT CTG CTG GGA CCG GCA GAA GCT GAA GGG TCT CAG 1059
Gly Glu Ala Gln Cys Leu Leu Gly Pro Ala Glu Ala Glu Gly Ser Gln
335 340 345

AGG AGG AGG CTG CTG GTT CCA GCA AAT GGT GCT GAC CCC ACT GAG ACT 1107
Arg Arg Arg Leu Leu Val Pro Ala Asn Gly Ala Asp Pro Thr Glu Thr
350 355 360

CTG ATG CTG TTC TTT GAC AAG TTT GCA AAC ATC GTG CCC TTT GAC TCC 1155
Leu Met Leu Phe Phe Asp Lys Phe Ala Asn Ile Val Pro Phe Asp Ser
365 370 375

TGG GAC CAG CTC ATG AGG CAG CTG GAC CTC ACG AAA AAT GAG ATC GAT 1203
Trp Asp Gln Leu Met Arg Gln Leu Asp Leu Thr Lys Asn Glu Ile Asp
380 385 390 395

GTG GTC AGA GCT GGT ACA GCA GGC CCA GGG GAT GCC TTG TAT GCA ATG 1251
Val Val Arg Ala Gly Thr Ala Gly Pro Gly Asp Ala Leu Tyr Ala Met
400 405 410

CTG ATG AAA TGG GTC AAC AAA ACT GGA CGG AAC GCC TCG ATC CAC ACC 1299
Leu Met Lys Trp Val Asn Lys Thr Gly Arg Asn Ala Ser Ile His Thr
415 420 425

CTG CTG GAT GCC TTG GAG AGG ATG GAA GAG AGA CAT GCA AAA GAG AAG 1347
Leu Leu Asp Ala Leu Glu Arg Met Glu Glu Arg His Ala Lys Glu Lys
430 435 440

ATT CAG GAC CTC TTG GTG GAC TCT GGA AAG TTC ATC TAC TTA GAA GAT 1395
Ile Gln Asp Leu Leu Val Asp Ser Gly Lys Phe Ile Tyr Leu Glu Asp
445 450 455

GGC ACA GGC TCT GCC GTG TCC TTG GAG TGAAAGACTC TTTTTACCAG 1442
Gly Thr Gly Ser Ala Val Ser Leu Glu
460 465

AGGTTTCCTC TTAGGTGTTA GGAGTTAATA CATATTAGGT TTTTTTTTTT TTTAACATGT 1502

ATACAAAGTA AATTCTTAGC CACGTGTATT GGCTCCTGCC TGTAATCCCA TCACTTTGGG 1562

AGGCTGACGC CGGTGGATCC ACTTGAGGTC CGAAGTTCCA AGACCAGCCC TGAACCAACA 1622

TCGTGGAAAT GCCCGTCTTT TACAAAAAAA TACCAAAAAT TCAACTGGAA TGTGCATGGT 1682

GTGTGCCATC ATTTCCTCGG CTAACTACGG GAGGTCTGAG GCCAGGAGAA TCCACTTGAA 1742

CCCCACGAAG GACAGTGTAG ACTGCAGATT GCACCACTGC ACTCCCAGCC TGGGAACACA 1802

GAGCAAGACT CTGTCTCAAG ATAAAATAAA ATAAACTTGA AAGAATTATT GCCCGACTGA 1862

GGCTCACATG CCAAAGGAAA ATCTGGTTCT CCCCTGAGCT GGCCTCCGTG TGTTTCCTTA 1922

TCATGGTGGT CAATTGGAGG TGTTAATTTG AATGGATTAA GGAACACCTA GAACACTGGT 1982

AAGGCATTAT TTCTGGGACA TTATTTCTGG GCATGTCTTC GAGGGTGTTT CCAGAGGGGA 2042

TTGGCATGCG ATCGGGTGGA CTGAGTGGAA AAGACCTACC CTTAATTTGG GGGGGCACCG 2102

TCCGACAGAC TGGGGAGCAA GATAGAAGAA AACAAAAAAA AAAAAAAAAA 2152

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Pro Pro Pro Ala Arg Val His Leu Gly Ala Phe Leu Ala Val
1 5 10 15

Thr Pro Asn Pro Gly Ser Ala Ala Ser Gly Thr Glu Ala Ala Ala Ala
20 25 30

Thr Pro Ser Lys Val Trp Gly Ser Ser Ala Gly Arg Ile Glu Pro Arg
35 40 45

Gly Gly Gly Arg Gly Ala Leu Pro Thr Ser Met Gly Gln His Gly Pro
50 55 60

Ser Ala Arg Ala Arg Ala Gly Arg Ala Pro Gly Pro Arg Pro Ala Arg
65 70 75 80

Glu Ala Ser Pro Arg Leu Arg Val His Lys Thr Phe Lys Phe Val Val
85 90 95

Val Gly Val Leu Leu Gln Val Val Pro Ser Ser Ala Ala Thr Ile Lys
100 105 110

Leu His Asp Gln Ser Ile Gly Thr Gln Gln Trp Glu His Ser Pro Leu

115 120 125

Gly Glu Leu Cys Pro Pro Gly Ser His Arg Ser Glu Arg Pro Gly Ala
130 135 140

Cys Asn Arg Cys Thr Glu Gly Val Gly Tyr Thr Asn Ala Ser Asn Asn
145 150 155 160

Leu Phe Ala Cys Leu Pro Cys Thr Ala Cys Lys Ser Asp Glu Glu Glu
165 170 175

Arg Ser Pro Cys Thr Thr Thr Arg Asn Thr Ala Cys Gln Cys Lys Pro
180 185 190

Gly Thr Phe Arg Asn Asp Asn Ser Ala Glu Met Cys Arg Lys Cys Ser
195 200 205

Thr Gly Cys Pro Arg Gly Met Val Lys Val Lys Asp Cys Thr Pro Trp
210 215 220

Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Asn Gly His Asn Ile
225 230 235 240

Trp Val Ile Leu Val Val Thr Leu Val Val Pro Leu Leu Leu Val Ala
245 250 255

Val Leu Ile Val Cys Cys Cys Ile Gly Ser Gly Cys Gly Gly Asp Pro
260 265 270

Lys Cys Met Asp Arg Val Cys Phe Trp Arg Leu Gly Leu Leu Arg Gly
275 280 285

Pro Gly Ala Glu Asp Asn Ala His Asn Glu Ile Leu Ser Asn Ala Asp
290 295 300

Ser Leu Ser Thr Phe Val Ser Glu Gln Gln Met Glu Ser Gln Glu Pro
305 310 315 320

Ala Asp Leu Thr Gly Val Thr Val Gln Ser Pro Gly Glu Ala Gln Cys
325 330 335

Leu Leu Gly Pro Ala Glu Ala Glu Gly Ser Gln Arg Arg Arg Leu Leu
340 345 350

Val Pro Ala Asn Gly Ala Asp Pro Thr Glu Thr Leu Met Leu Phe Phe
355 360 365

Asp Lys Phe Ala Asn Ile Val Pro Phe Asp Ser Trp Asp Gln Leu Met
370 375 380

Arg Gln Leu Asp Leu Thr Lys Asn Glu Ile Asp Val Val Arg Ala Gly
385 390 395 400

Thr Ala Gly Pro Gly Asp Ala Leu Tyr Ala Met Leu Met Lys Trp Val
405 410 415

Asn Lys Thr Gly Arg Asn Ala Ser Ile His Thr Leu Leu Asp Ala Leu
420 425 430

Glu Arg Met Glu Glu Arg His Ala Lys Glu Lys Ile Gln Asp Leu Leu
435 440 445

Val Asp Ser Gly Lys Phe Ile Tyr Leu Glu Asp Gly Thr Gly Ser Ala
450 455 460

Val Ser Leu Glu
465

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 669 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala
1 5 10 15

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
20 25 30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn
35 40 45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
50 55 60

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65 70 75 80

Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
85 90 95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
100 105 110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
115 120 125

Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
130 135 140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145 150 155 160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
165 170 175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
180 185 190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
195 200 205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu
210 215 220

```
Ser Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met
225                 230                 235                 240

Thr Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu
                245                 250                 255

Ala Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu
            260                 265                 270

Gln Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys
        275                 280                 285

Glu Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys
    290                 295                 300

Thr Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser
305                 310                 315                 320

Asp Ser Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val Met
                325                 330                 335

Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala Arg
            340                 345                 350

Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser Lys
        355                 360                 365

Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn Leu
    370                 375                 380

Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro Pro
385                 390                 395                 400

Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro Asp
                405                 410                 415

Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His Phe
            420                 425                 430

Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly Leu
        435                 440                 445

Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg Cys
    450                 455                 460

Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp Pro
465                 470                 475                 480

Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr Ser
                485                 490                 495

Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp Leu
            500                 505                 510

Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Val Lys Arg Lys Glu
        515                 520                 525

Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly Ser His
    530                 535                 540

Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu Ser Asp
545                 550                 555                 560
```

Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met Thr Leu
565 570 575

Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu Ala Lys
580 585 590

Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu Gln Lys
595 600 605

Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys Glu Ala
610 615 620

Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys Thr Leu
625 630 635 640

Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser Asp Ser
645 650 655

Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
660 665

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 909 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Pro Leu Val Leu Leu Glu
1 5 10 15

Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro His
20 25 30

Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys Tyr
35 40 45

Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly
50 55 60

Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Asp Thr Asp Cys Arg
65 70 75 80

Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His
85 90 95

Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile
100 105 110

Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn
115 120 125

Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys
130 135 140

Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln
145 150 155 160

Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu
165 170 175

Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu
180 185 190

Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr
195 200 205

Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu Leu Ser
210 215 220

Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys Ser Lys
225 230 235 240

Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu Gly Glu
245 250 255

Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser Phe Ser
260 265 270

Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val Pro Ser
275 280 285

Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys Pro Asn
290 295 300

Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly Ala Asp
305 310 315 320

Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn Pro Leu
325 330 335

Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp Thr Asp
340 345 350

Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro Leu Arg
355 360 365

Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu Ile Asp
370 375 380

Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln Tyr Ser
385 390 395 400

Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala Thr Leu
405 410 415

Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly Cys Leu
420 425 430

Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro Pro Ala
435 440 445

Pro Ser Leu Leu Arg Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu
450 455 460

Pro Leu Val Leu Leu Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val
465 470 475 480

Ile Gly Leu Val Pro His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val
485 490 495

Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys
500 505 510

Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro
515 520 525

Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala
530 535 540

Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Glu
545 550 555 560

Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp
565 570 575

Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu
580 585 590

Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val
595 600 605

His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala
610 615 620

Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys
625 630 635 640

Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val
645 650 655

Lys Gly Thr Glu Asp Ser Gly Thr Thr Val Leu Leu Pro Leu Val Ile
660 665 670

Phe Phe Gly Leu Cys Leu Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr
675 680 685

Arg Tyr Gln Arg Trp Lys Ser Asp Leu Tyr Ser Ile Val Cys Gly Lys
690 695 700

Ser Thr Pro Glu Lys Glu Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro
705 710 715 720

Leu Ala Pro Asn Pro Ser Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr
725 730 735

Leu Gly Phe Ser Pro Val Pro Ser Ser Thr Phe Thr Ser Ser Ser Thr
740 745 750

Tyr Thr Pro Gly Asp Cys Pro Asn Phe Ala Ala Pro Arg Arg Glu Val
755 760 765

Ala Pro Pro Tyr Gln Gly Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala
770 775 780

Ser Asp Pro Ile Pro Asn Pro Leu Gln Lys Trp Glu Asp Ser Ala His
785 790 795 800

Lys Pro Gln Ser Leu Asp Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val
805 810 815

```
Val Glu Asn Val Pro Pro Leu Arg Trp Lys Glu Phe Val Arg Arg Leu
            820                 825                 830

Gly Leu Ser Pro His Glu Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg
        835                 840                 845

Cys Leu Arg Glu Ala Gln Tyr Ser Met Leu Ala Thr Trp Arg Arg Arg
    850                 855                 860

Thr Pro Arg Arg Glu Ala Thr Leu Glu Leu Leu Gly Arg Val Leu Arg
865                 870                 875                 880

Asp Met Asp Leu Leu Gly Cys Leu Glu Asp Ile Glu Glu Ala Leu Cys
                885                 890                 895

Gly Pro Ala Ala Leu Pro Pro Ala Pro Ser Leu Leu Arg
            900                 905
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 833 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Glu Gln Arg Pro Arg Gly Cys Ala Ala Val Ala Ala Ala Leu Leu
1               5                   10                  15

Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
            20                  25                  30

Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
        35                  40                  45

Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
    50                  55                  60

Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
65                  70                  75                  80

Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
                85                  90                  95

Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
            100                 105                 110

Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
        115                 120                 125

Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
    130                 135                 140

Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
145                 150                 155                 160
```

```
Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
                165                 170                 175

Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
            180                 185                 190

Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
        195                 200                 205

Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
    210                 215                 220

Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
225                 230                 235                 240

Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp
                245                 250                 255

Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
            260                 265                 270

Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
        275                 280                 285

Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
    290                 295                 300

Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
305                 310                 315                 320

Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
                325                 330                 335

Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
            340                 345                 350

Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
        355                 360                 365

Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
    370                 375                 380

Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
385                 390                 395                 400

Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
                405                 410                 415

Pro Met Glu Gln Arg Pro Arg Gly Cys Ala Ala Val Ala Ala Ala Leu
            420                 425                 430

Leu Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro
        435                 440                 445

Arg Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys
    450                 455                 460

Cys Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu
465                 470                 475                 480

Pro Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu
                485                 490                 495
```

```
Ala Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys
            500                 505                 510

Asp Glu Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
        515                 520                 525

Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
    530                 535                 540

Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
545                 550                 555                 560

Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
                565                 570                 575

Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
            580                 585                 590

Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
        595                 600                 605

Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
    610                 615                 620

Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
625                 630                 635                 640

Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
                645                 650                 655

Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp
            660                 665                 670

Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
        675                 680                 685

Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
    690                 695                 700

Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
705                 710                 715                 720

Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
                725                 730                 735

Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
            740                 745                 750

Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
        755                 760                 765

Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
    770                 775                 780

Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
785                 790                 795                 800

Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
                805                 810                 815

Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
            820                 825                 830
```

Pro

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 426 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GGCANAGGTN CGTACCTAGC TCACCTGCAA CCATCAAACT TNATGATCAA TCAATTGGCA    60
CACAGCAATG GGAAACATAG CCCTTTGGAA GANTTGTNTC CACCAGGATC TCATAGATCA   120
AAACATCCTG GGAGCCTGTT AACCGGTGCC CCAAAGGNTG GTCAAGGTCA AGGAATTGTT   180
NCGCCCTGGA AGTGAACATC GAGTGTNTCC ACAAAGGATT CAGGCAATGG GACATAAATA   240
TATGGGTGAA TTTTGGTTGT GAACTTTGGT TGNTCCCGTT GNTGTTGNTG GCTGTGCTGA   300
TTGTTTGTTG TTGCATCGGC TTCAGGTTNT GGAGGGGGAC CCAAGTGCAT GGACAGGGTG   360
TGTTTCTGGG GTTTGGGTCT CTTAGAGGGC NTGGGTTANG GCANGTTCAC AAGGGTTTTA   420
GCAANG                                                              426
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
TGGGGCTGAG GACAATGCTG ACNACGAGAT TCTGAGCAAC GCAGNACTNG CTGTCCACTT    60
TCGTCTNTGN GCAGCAAATG GAAAGCCAGG AGCCGGCAGA TTTGACAGGT GTCACTGTAC   120
AGTCCCCAGG GGAGGCACAG TGTCTGCTGG TGAGTTGGGG ACAGGCCCTT GCAAGACCTT   180
GTGAGGCAGG GGGTGAAGGC CATGNCTCGG CTTCNNNTGG TCAAAGGGGA AGTGGAGCCT   240
GAGGGAGATG GGACTTNAGG GGGACGGNGC TGCGTGGGGA AAAAGCAGCC ACCNTTTGAC   300
AAGGGGGACA GGCATTTTTN CAAATGTGTG CTTNTTGGT                          339
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGGCATGCA TGATCAATCA ATTGGCAC 28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCGGGATCCG CCATCATGGC GCCACCACCA GCTAGA 36

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCGGGATCCT CACTCCAAGG ACACGGCAGA GCC 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCGGGATCCT CAATTATGTC
CATTGCCTG 29

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGAAGCTTT

CAATTATGTC CATTGCCTG 29